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(54) Title: ALZHEIMER'S DISEASE SECRETASE, APP SUBSTRATES THEREFOR, AND USES THEREFOR

(57) Abstract: The present invention provides the enzyme and enzymatic procedures for cleaving the β secretase cleavage site of the APP protein and associated nucleic acids, peptides, vectors, cells and isolates and assays. An enzyme that cleaves the α-secretase site of APP also is provided. The invention further provides a modified APP protein and associated nucleic acids, peptides, vectors, cells, and cell isolates, and assays that are particularly useful for identifying candidate therapeutics for treatment or prevention of Alzheimer's disease.

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Alzheimer's Disease Secretase, APP Substrates Therefor, and Uses Therefor

The present application is a continuation-in-part of United States Patent Application 09/416,901, filed October 13, 1999 which claims priority benefit of United States Provisional Patent Application No. 60/155,493, filed September 23, 1999 and United States Provisional Patent Application 60/169,232, filed December 6, 1999. The present application also claims priority benefit as a continuation-in-part of United States Patent Application Serial No. 09/404,133 and PCT/US99/20881, both filed September 23, 1999, both of which in turn claim priority benefit of United States Provisional Patent Application No. 60/101,594, filed September 24, 1998. All of these priority applications are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to Alzheimer's Disease, amyloid protein precursor, amyloid beta peptide, and human aspartyl proteases, as well as a method for the identification of agents that modulate the activity of these polypeptides and thereby are candidates to modulate the progression of Alzheimer's disease.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) causes progressive dementia with consequent formation of amyloid plaques, neurofibrillary tangles, gliosis and neuronal loss. The disease occurs in both genetic and sporadic forms whose clinical course and pathological features are quite similar. Three genes have been discovered to date which, when mutated, cause an autosomal dominant form of Alzheimer's disease. These encode the amyloid protein precursor (APP) and two related proteins, presenilin-1 (PS1) and presenilin-2 (PS2), which, as their names suggest, are structurally and functionally related. Mutations in any of the three proteins have been observed to enhance proteolytic processing of APP via an intracellular pathway that produces amyloid beta peptide (Aβ peptide, or sometimes here as Abeta), a 40-42 amino acid long peptide that is the primary component of amyloid plaque in AD.

Dysregulation of intracellular pathways for proteolytic processing may be central to the pathophysiology of AD. In the case of plaque formation, mutations in APP, PS1 or PS2 consistently alter the proteolytic processing of APP so as to enhance formation of Aβ 1-42, a form of the Aβ peptide which seems to be particularly amyloidogenic, and thus very important in AD. Different forms of APP range in size from 695-770 amino acids, localize to the cell surface, and have a single C-terminal transmembrane domain. Examples of specific isotypes of APP which are currently known to exist in humans are the 695-amino acid polypeptide described by Kang *et. al.* (1987), *Nature 325*: 733-736 which is designated as the "normal" APP; the 751 amino acid polypeptide described by Ponte *et al.* (1988), *Nature 331*: 525-527 (1988) and Tanzi *et al.* (1988), *Nature 331*: 528-530; and the 770 amino acid polypeptide described by Kitaguchi *et. al.* (1988), *Nature 331*: 530-532. The Abeta peptide is derived from a region of APP adjacent to and containing a portion of the transmembrane domain. Normally, processing of APP at the α-secretase site cleaves the midregion of the Aβ sequence adjacent to the membrane and releases the soluble, extracellular domain of APP from the cell surface. This α-secretase APP processing creates soluble APP- α, (sAPPα) which is normal and not thought to contribute to AD.

Pathological processing of APP at the β- and γ-secretase sites, which are located N-terminal and C-terminal to the α-secretase site, respectively, produces a very different result than processing at the α site. Sequential processing at the β- and γ-secretase sites releases the Aβ peptide, a peptide possibly very important in AD pathogenesis. Processing at the β- and γ-secretase sites can occur in both the endoplasmic reticulum (in neurons) and in the endosomal/lysosomal pathway after reinternalization of cell surface APP (in all cells). Despite intense efforts, for 10 years or more, to identify the enzymes responsible for processing APP at the β and γ sites, to produce the Aβ peptide, those proteases remained unknown until this disclosure.

SUMMARY OF THE INVENTION

Here, for the first time, we report the identification and characterization of the β secretase enzyme, termed Aspartyl Protease 2 (Asp2). We disclose some known and some novel human aspartic proteases that can act as β-secretase proteases and, for the first time, we explain the role these proteases have in AD. We describe regions in the proteases critical for their unique function and for the first time characterize their substrate. This is the first description of expressed isolated purified active protein of this type, assays that use the protein, in addition to the identification and creation of useful cell lines and inhibitors. We also identify and characterize both α-secretase and β-secretase activities of a protease, designated as Asp1.

Here we disclose a number of variants of the Asp2 gene and peptide.

In one aspect, the invention provides any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for the peptide DTG, where the first nucleic acid of the first special set of nucleic acids is the first special nucleic acid, and where the second set of nucleic acids code for either the peptide DSG or DTG, where the last nucleic acid of the second set of nucleic acids is the last special nucleic acid, with the proviso that the nucleic acids disclosed in SEQ ID NO. 1 and SEQ ID NO. 3 are not included. In a preferred embodiment, the two sets of special nucleic acids are separated by nucleic acids that code for about 125 to 222 amino acid positions, which may be any amino acids. In a highly preferred embodiment, the two sets of special nucleic acids are separated by nucleic acids that code for about 150 to 196, or 150-190, or 150 to 172 amino acid positions, which may be any amino acids. In a particular preferred embodiment, the two sets are separated by nucleic acids that code for about 172 amino acid positions, which may be any amino acids. An exemplary nucleic acid polynucleotide comprises the acid nucleotide sequence in SEQ ID NO. 5. In another particular preferred

embodiment, the two sets are separated by nucleic acids that code for about 196 amino acids. An exemplary polynucleotide comprises the nucleotide sequence in SEQ ID NO. 5. In another particular embodiment, the two sets of nucleotides are separated by nucleic acids that code for about 190 amino acids. An exemplary polynucleotide comprises the nucleotide sequence in SEQ ID NO. 1. Preferably, the first nucleic acid of the first special set of amino acids, that is, the first special nucleic acid, is operably linked to any codon where the nucleic acids of that codon codes for any peptide comprising from 1 to 10,000 amino acid (positions). In one variation, the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: any reporter proteins or proteins which facilitate purification. For example, the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathione S transferase, Green Fluorescent protein, and ubiquitin. In another variation, the last nucleic acid of the second set of special amino acids, that is, the last special nucleic acid, is operably linked to nucleic acid polymers that code for any peptide comprising any amino acids from 1 to 10,000 amino acids. In still another variation, the last special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: any reporter proteins or proteins which facilitate purification. For example, the last special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathione S transferase, Green Fluorescent protein, and ubiquitin.

In a related aspect, the invention provides any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for DTG, where the first nucleic acid of the first special set of nucleic acids is the first

special nucleic acid, and where the second set of nucleic acids code for either DSG or DTG, where the last nucleic acid of the second set of special nucleic acids is the last special nucleic acid, where the first special nucleic acid is operably linked to nucleic acids that code for any number of amino acids from zero to 81 amino acids and where each of those codons may code for any amino acid. In a preferred embodiment, the first special nucleic acid is operably linked to nucleic acids that code for any number of from 64 to 77 amino acids where each codon may code for any amino acid. In a particular embodiment, the first special nucleic acid is operably linked to nucleic acids that code for 71 amino acids. For example, the first special nucleic acid is operably linked to 71 amino acids and where the first of those 71 amino acids is the amino acid T. In a preferred embodiment, the polynucleotide comprises a sequence that is at least 95% identical to a human Asp1 or Asp2 sequence as taught herein. In another preferred embodiment, the first special nucleic acid is operably linked to nucleic acids that code for any number of from 30 to 54 amino acids, or 35 to 47 amino acids, or 40 to 54 amino acids where each codon may code for any amino acid. In a particular embodiment, the first special nucleic acid is operably linked to nucleic acids that code for 47 amino acids. For example, the first special nucleic acid is operably linked to 47 codons where the first those 47 amino acids is the amino acid E.

In another related aspect, the invention provides for any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta (β) secretase cleavage site of APP and that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for the peptide DTG, where the first nucleic acid of the first special set of amino acids is, the first special nucleic acid, and where the second set of special nucleic acids code for either the peptide DSG or DTG, where the last nucleic acid of the second set of special nucleic acids, the last special nucleic acid, is operably linked to nucleic acids that code for any number of codons from 50 to 170 codons. In a preferred embodiment, the last special nucleic acid is operably linked to nucleic acids

comprising from 100 to 170 codons. In a highly preferred embodiment, the last special nucleic acid is operably linked to nucleic acids comprising from 142 to 163 codons. In a particular embodiment, the last special nucleic acid is operably linked to nucleic acids comprising about 142 codons, or about 163 codons, or about 170 codons. In a highly preferred embodiment, the polynucleotide comprises a sequence that is at least 95% identical to aspartyl-protease encoding sequences taught herein. In one variation, the second set of special nucleic acids code for the peptide DSG. In another variation, the first set of nucleic acid polynucleotide is operably linked to a peptide purification tag. For example, the nucleic acid polynucleotide is operably linked to a peptide purification tag which is six histidine. In still another variation, the first set of special nucleic acids are on one polynucleotide and the second set of special nucleic acids are on a second polynucleotide, where both first and second polynucleotides have at lease 50 codons. In one embodiment of this type, both of the polynucleotides are in the same solution. In a related aspect, the invention provides a vector which contains a polynucleotide as described above, or a cell or cell line which is transformed or transfected with a polynucleotide as described above or with a vector containing such a polynucleotide.

In still another aspect, the invention provides an isolated or purified peptide or protein comprising an amino acid polymer that is a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid position can be any amino acid, where the first set of special amino acids consists of the peptide DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, where the second set of amino acids is selected from the peptide comprising either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, with the proviso that the proteases disclosed in SEQ ID NO. 2 and SEQ ID NO. 4 are not included. In preferred embodiments, the two sets of amino acids are separated by about 125 to 222 amino acid positions or about 150 to 196 amino acids, or about 150-190 amino acids, or about 150 to 172 amino acids, where in each position it may be

any amino acid. In a particular embodiment, the two sets of amino acids are separated by about 172 amino acids. For example, the protease has the amino acid sequence described in SEQ ID NO 6. In another particular embodiment, the two sets of amino acids are separated by about 196 amino acids. For example, the two sets of amino acids are separated by the same amino acid sequences that separate the same set of special amino acids in SEQ ID NO 4. In another particular embodiment, the two sets of nucleotides are separated by about 190 amino acids. For example, the two sets of nucleotides are separated by the same amino acid sequences that separate the same set of special amino acids in SEQ ID NO 2. In one embodiment, the first amino acid of the first special set of amino acids, that is, the first special amino acid, is operably linked to any peptide comprising from 1 to 10,000 amino acids. In another embodiment, the first special amino acid is operably linked to any peptide selected from the group consisting of: any reporter proteins or proteins which facilitate purification. In particular embodiments, the first special amino acid is operably linked to any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathione S transferase, Green Fluorescent protein, and ubiquitin. In still another variation, the last amino acid of the second set of special amino acids, that is, the last special amino acid, is operably linked to any peptide comprising any amino acids from 1 to 10,000 amino acids. By way of nonlimiting example, the last special amino acid is operably linked any peptide selected from the group consisting of any reporter proteins or proteins which facilitate purification. In particular embodiments, the last special amino acid is operably linked to any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathione S transferase, Green Fluorescent protein, and ubiquitin.

In a related aspect, the invention provides any isolated or purified peptide or protein comprising an amino acid polypeptide that codes for a protease capable of cleaving the beta secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid in each position can be any amino acid, where the first set of special amino acids consists of the amino acids DTG, where the

first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids is either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, where the first special amino acid is operably linked to amino acids that code for any number of amino acids from zero to 81 amino acid positions where in each position it may be any amino acid. In a preferred embodiment, the first special amino acid is operably linked to a peptide from about 30-77 or about 64 to 77 amino acids positions where each amino acid position may be any amino acid. In a particular embodiment, the first special amino acid is operably linked to a peptide 35, 47, 71, or 77 amino acids. In a very particular embodiment, the first special amino acid is operably linked to 71 amino acids and the first of those 71 amino acids is the amino acid T. For example, the polypeptide comprises a sequence that is at least 95% identical to an aspartyl protease sequence as described herein. In another embodiment, the first special amino acid is operably linked to any number of from 40 to 54 amino acids (positions) where each amino acid position may be any amino acid. In a particular embodiment, the first special amino acid is operably linked to amino acids that code for a peptide of 47 amino acids. In a very particular embodiment, the first special amino acid is operably linked to a 47 amino acid peptide where the first those 47 amino acids is the amino acid E. In another particular embodiment, the first special amino acid is operably linked to the same corresponding peptides from SEQ ID NO. 3 that are 35, 47, 71, or 77 peptides in length, beginning counting with the amino acids on the first special sequence, DTG, towards the N-terminal of SEQ ID NO. 3. In another particular embodiment, the polypeptide comprises a sequence that is at least 95% identical to the same corresponding amino acids in SEQ ID NO. 4, that is, identical to that portion of the sequences in SEQ ID NO. 4, including all the sequences from both the first and or the second special nucleic acids, toward the – terminal, through and including 71, 47, 35 amino acids before the first special amino acids. For example, the complete polypeptide comprises the peptide of 71 amino acids, where the first of the amino acid is T and the second is Q.

In still another related aspect, the invention provides any isolated or purified amino acid polypeptide that is a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid in each position can be any amino acid, where the first set of special amino acids consists of the amino acids that code for DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids are either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, which is operably linked to any number of amino acids from 50 to 170 amino acids, which may be any amino acids. In preferred embodiments, the last special amino acid is operably linked to a peptide of about 100 to 170 amino acids or about 142-163 amino acids. In particular embodiments, the last special amino acid is operably linked to a peptide of about 142 amino acids, or about 163 amino acids, or about 170 amino acids. For example, the polypeptide comprises a sequence that is at least 95% identical (and preferably 100% identical) to an aspartyl protease sequence as described herein. In one particular embodiment, the second set of special amino acids is comprised of the peptide with the amino acid sequence DSG. Optionally, the amino acid polypeptide is operably linked to a peptide purification tag, such as purification tag which is six histidine. In one variation, the first set of special amino acids are on one polypeptide and the second set of special amino acids are on a second polypeptide, where both first and second polypeptide have at lease 50 amino acids, which may be any amino acids. In one embodiment of this type, both of the polypeptides are in the same vessel. The invention further includes a process of making any of the polynucleotides, vectors, or cells described herein; and a process of making any of the polypeptides described herein.

In yet another related aspect, the invention provides a purified polynucleotide comprising a nucleotide sequence that encodes a polypeptide having aspartyl protease activity, wherein the polypeptide has an amino acid sequence characterized by: (a) a first tripeptide sequence DTG; (b) a second tripeptide sequence selected from the

group consisting of DSG and DTG; and (c) about 100 to 300 amino acids separating the first and second tripeptide sequences, wherein the polypeptide cleaves the beta secretase cleavage site of amyloid protein precursor. In one embodiment, the polypeptide comprises an amino acid sequence depicted in SEQ ID NO: 2 or 4, whereas in another embodiment, the polypeptide comprises an amino acid sequence other than the amino acid sequences set forth in SEQ ID NOs: 2 and 4. Similarly, the invention provides a purified polynucleotide comprising a nucleotide sequence that encodes a polypeptide that cleaves the beta secretase cleavage site of amyloid protein precursor; wherein the polynucleotide includes a strand that hybridizes to one or more of SEQ ID NOs: 3, 5, and 7 under the following hybridization conditions: hybridization overnight at 42°C for 2.5 hours in 6 X SSC/0.1% SDS, followed by washing in 1.0 X SSC at 65°C, 0.1% SDS. In one embodiment, the polypeptide comprises an amino acid sequence depicted in SEQ ID NO: 2 or 4, whereas in another embodiment, the polypeptide comprises an amino acid sequence other than the amino acid sequences set forth in SEQ ID NOs: 2 and 4. Likewise, the invention provides a purified polypeptide having aspartyl protease activity, wherein the polypeptide is encoded by polynucleotides as described in the preceding sentences. The invention also provides a vector or host cell comprising such polynucleotides, and a method of making the polypeptides using the vectors or host cells to recombinantly express the polypeptide.

In yet another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide, said polynucleotide encoding a Hu-Asp polypeptide and having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), wherein said Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) polypeptides have the complete amino acid sequence of SEQ ID NO. 2, SEQ ID NO. 4, and SEQ ID NO. 6, respectively; and

(b) a nucleotide sequence complementary to the nucleotide sequence of (a).

Several species are particularly contemplated. For example, the invention provides a nucleic acid and molecule wherein said Hu-Asp polypeptide is Hu-Asp1, and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO. 1; and a nucleic acid molecule wherein said Hu-Asp polypeptide is Hu-Asp2(a), and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO. 4; and a nucleic acid molecule wherein said Hu-Asp polypeptide is Hu-Asp2(b), and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO. 5. In addition to the foregoing, the invention provides an isolated nucleic acid molecule comprising polynucleotide which hybridizes under stringent conditions to a polynucleotide having the nucleotide sequence in (a) or (b) as described above.

Additionally, the invention provides a vector comprising a nucleic acid molecule as described in the preceding paragraph. In a preferred embodiment, the nucleic acid molecule is operably linked to a promoter for the expression of a Hu-Asp polypeptide. Individual vectors which encode Hu-Asp1, and Hu-Asp2(a), and Hu-Asp2(b) are all contemplated. Likewise, the invention contemplates a host cell comprising any of the foregoing vectors, as well as a method of obtaining a Hu-Asp polypeptide comprising culturing such a host cell and isolating the Hu-Asp polypeptide. Host cells of the invention include bacterial cells, such as *E. coli*, and eukaryotic cells. Among the eukaryotic cells that are contemplated are insect cells, such as sf9 or High 5 cells; and mammalian cells, such as human, rodent, lagomorph, and primate. Preferred human cells include HEK293, and IMR-32 cells. Other preferred mammalian cells include COS-7, CHO-K1, Neuro-2A, and 3T3 cells. Also among the eukaryotic cells that are contemplated are a yeast cell and an avian cell.

In a related aspect, the invention provides an isolated Hu-Asp1 polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO. 2. The invention also provides an isolated Hu-Asp2(a) polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO. 4. The invention also provides an isolated Hu-Asp2(a) polypeptide comprising an amino acid sequence at

least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO. 8.

In still another aspect, the invention provides an isolated antibody that binds specifically to any Hu-Asp polypeptide described herein, especially the polypeptide described in the preceding paragraphs.

The invention also provides several assays involving aspartyl protease enzymes of the invention. For example, the invention provides

a method to identify a cell that can be used to screen for inhibitors of β secretase activity comprising:

(a) identifying a cell that expresses a protease capable of cleaving APP at the β secretase site, comprising:

i) collect the cells or the supernatant from the cells to be identified

ii) measure the production of a critical peptide, where the critical peptide is selected from the group consisting of either the APP C-terminal peptide or soluble APP,

iii) select the cells which produce the critical peptide.

In one variation, the cells are collected and the critical peptide is the APP C-terminal peptide created as a result of the β secretase cleavage. In another variation, the supernatant is collected and the critical peptide is soluble APP, where the soluble APP has a C-terminus created by β secretase cleavage. In preferred embodiments, the cells contain any of the nucleic acids or polypeptides described above and the cells are shown to cleave the β-secretase site of any peptide having the following peptide structure, P2, P1, P1', P2', where P2 is K or N, where P1 is M or L, where P1' is D, where P2' is A. The method where P2 is K and P1 is M. The method where P2 is N and P1 is L.

In still another aspect, the invention provides novel isoforms of amyloid protein precursor (APP) where the last two carboxy terminus amino acids of that isoform are both lysine residues. In this context, the term "isoform" is defined as any APP polypeptide, including APP variants (including mutations), and APP fragments

that exists in humans, such as those described in US 5,766,846, col 7, lines 45-67, incorporated into this document by reference, modified as described herein by the inclusion of two C-terminal lysine residues. For example, the invention provides a polypeptide comprising the isoform known as APP695, modified to include two lysine residues as its last two carboxy terminus amino acids. An exemplary polypeptide comprises the amino acid sequence set forth in SEQ ID NO. 16. The invention further includes APP isoform variants as set forth in SEQ ID NOs. 18 and 20. The invention further includes all polynucleotides that encode an APP protein that has been modified to include two C-terminal lysines; as well has any eukaryotic cell line comprising such nucleic acids or polypeptides. Preferred cell lines include a mammalian cell line (*e.g.*, HEK293, Neuro2a).

Thus, in one embodiment, the invention provides a polypeptide comprising the amino acid sequence of a mammalian amyloid protein precursor (APP) or fragment thereof containing an APP cleavage site recognizable by a mammalian β-secretase, and further comprising two lysine residues at the carboxyl terminus of the amino acid sequence of the mammalian APP or APP fragment. As taught herein in detail, the addition of two additional lysine residues to APP sequences has been found to greatly increase Aβ processing of the APP in APP processing assays. Thus, the di-lysine modified APP reagents of the invention are particularly useful in assays to identify modulators of Aβ production, for use in designing therapeutics for the treatment or prevention of Alzheimer's disease. In one embodiment, the polypeptide comprises the complete amino acid sequence of a mammalian amyloid protein precursor (APP), and further comprises the two lysine residues at the carboxyl terminus of the amino acid sequence of the mammalian amyloid protein precursor. In an alternative embodiment, the polypeptide comprises only a fragment of the APP, the fragment containing at least that portion of APP that is cleaved by a mammalian β-secretase (or α-secretase or γ-secretase) in the formation of Aβ peptides.

The practice of assays that monitor cleavage of APP can be facilitated by attaching a marker to a portion of the APP. Measurment of retained or liberated marker can be used to quantitate the amount of APP cleavage that occurs in the assay,

e.g., in the presence or absence of a putative modulator of cleavage activity. Thus, in one preferred embodiment, the polypeptide of the invention further includes a marker. For example, the marker comprises a reporter protein amino acid sequence attached to the APP amino acid sequence. Exemplary reporter proteins include a fluorescing protein (e.g., green fluorescing proteins, luciferase) or an enzyme that is used to cleave a substrate to produce a colorimetric cleavage product. Also contemplated are tag sequences which are commonly used as epitopes for quantitative immunoassays.

In a preferred embodiment, the di-lysine-modified APP of the invention is a human APP. For example, human APP isoforms such as APP695, APP751, and APP770, modified to include the two lysines, are contemplated. In a preferred embodiment, the APP isoform comprises at least one variation selected from the group consisting of a Swedish KM-NL mutation and a London V717-F mutation, or any other mutation that has been observed in a subpopulation that is particularly prone to development of Alzheimer's disease. These mutations are recognized as mutations that influence APP processing into Aβ. In a highly preferred embodiment, the APP protein or fragment thereof comprises the APP-Sw β-secretase peptide sequence NLDA, which is associated with increased levels of Aβ processing and therefore is particularly useful in assays relating to Alzheimer's research. More particularly, the APP protein or fragment thereof preferably comprises the APP-Sw β-secretase peptide sequence SEVNLDAEFR (SEQ ID NO: 63).

In one preferred embodiment, the APP protein or fragment thereof further includes an APP transmembrane domain carboxy-terminal to the APP-Sw β-secretase peptide sequence. Polypeptides that include the TM domain are particularly useful in cell-based APP processing assays. In contrast, embodiments lacking the TM domain are useful in cell-free assays of APP processing.

In addition to working with APP from humans and various animal models, researchers in the field of Alzheimer's also have construct chimeric APP polypeptides which include stretches of amino acids from APP of one species (e.g., humans) fused to streches of APP from one or more other species (e.g., rodent). Thus, in another embodiment of the polypeptide of the invention, the APP protein or fragment thereof

comprises a chimeric APP, the chimeric APP including partial APP amino acid sequences from at least two species. A chimeric APP that includes amino acid sequence of a human APP and a rodent APP is particularly contemplated.

In a related aspect, the invention provides a polynucleotide comprising a nucleotide sequence that encodes a polypeptide as described in the preceding paragraphs. Such a polynucleotide is useful for recominant expression of the polypeptide of the invention for use in APP processing assays. In addition, the polynucleotide is useful for transforming into cells to produce recombinant cells that express the polypeptide of the invention, which cells are useful in cell-based assays to identify modulators of APP processing. Thus, in addition to polynucleotides, the invention provides a vector comprising such polynucleotides, especially expression vectors where the polynucleotide is operably linked to a promoter to promote expression of the polypeptide encoded by the polynucleotide in a host cell. The invention further provides a host cell transformed or transfected with such a polynucleotide or a vector. Among the preferred host cells are mammalian cells, especially human cells.

In another, related embodiment, the invention provides a polypeptide useful for assaying for modulators of β-secretase activity, said polypeptide comprising an amino acid sequence of the formula NH_2-X-Y-Z-KK-COOH; wherein X, Y, and Z each comprise an amino acid sequence of at least one amino acid; wherein-NH_2-X comprises an amino-terminal amino acid sequence having at least one amino acid residue; wherein Y comprises an amino acid sequence of a β-secretase recognition site of a mammalian amyloid protein precursor (APP); and wherein Z-KK-COOH comprises a carboxy-terminal amino acid sequence ending in two lysine (K) residues. In one preferred variation, the carboxyl-terminal amino acid sequence Z includes a hyrdrophobic domain that is a transmembrane domain in host cells that express the polypeptide. Host cells that express such a polypeptide are particularly useful in assays described herein for identifying modulators of APP processing. In another preferred variation, the amino-terminal amino acid sequence X includes an amino acid sequence of a reporter or marker protein, as described above. In still another preferred

variation, the β-secretase recognition site Y comprises the human APP-Sw β-secretase peptide sequence NLDA. It will be apparent that these preferred variations are not mutually exclusive of each other -- they may be combined in a single polypeptide. The invention further provides a polynucleotide comprising a nucleotide sequence that encodes such polypeptides, vectors which comprise such polynucleotides, and host cells which comprises such vectors, polynucleotides, and/or polypeptides.

In yet another aspect, the invention provides a method for identifying inhibitors of an enzyme that cleaves the beta secretase cleavable site of APP comprising:

a) culturing cells in a culture medium under conditions in which the enzyme causes processing of APP and release of amyloid beta-peptide into the medium and causes the accumulation of CTF99 fragments of APP in cell lysates,

b) exposing the cultured cells to a test compound; and specifically determining whether the test compound inhibits the function of the enzyme by measuring the amount of amyloid beta-peptide released into the medium and/or the amount of CTF99 fragments of APP in cell lysates;

c) identifying test compounds diminishing the amount of soluble amyloid beta peptide present in the culture medium and diminution of CTF99 fragments of APP in cell lysates as Asp2 inhibitors. In preferred embodiments, the cultured cells are a human, rodent or insect cell line. It is also preferred that the human or rodent cell line exhibits β secretase activity in which processing of APP occurs with release of amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates. Among the contemplated test compounds are antisense oligomers directed against the enzyme that exhibits β secretase activity, which oligomers reduce release of soluble amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates.

In yet another aspect, the invention provides a method for the identification of an agent that decreases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method comprising:

a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent; whereby a lower level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has decreased the activity of said Hu-Asp polypeptide.

In a related aspect, the invention provides a method for assaying for modulators of β-secretase activity, comprising the steps of:

(a) contacting a first composition with a second composition both in the presence and in the absence of a putative modulator compound, wherein the first composition comprises a mammalian β-secretase polypeptide or biologically active fragment thereof, and wherein the second composition comprises a substrate polypeptide having an amino acid sequence comprising a β-secretase cleavage site; (b) measuring cleavage of the substrate polypeptide in the presence and in the absence of the putative modulator compound; and (c) identifying modulators of β-secretase activity from a difference in cleavage in the presence versus in the absence of the putative modulator compound. A modulator that is a β-secretase antagonist (inhibitor) reduces such cleavage, whereas a modulator that is a β-secretase agonist increases such cleavage. Since such assays are relevant to development of Alzheimer's disease therapeutics for humans, it will be readily apparent that, in one preferred embodiment, the first composition comprises a purified human Asp2 polypeptide. In one variation, the first composition comprises a soluble fragment of a human Asp2 polypeptide that retains Asp2 β-secretase activity. Several such fragments (including ΔTM fragments) are described herein in detail. Thus, in a particular embodiment, the soluble fragment is a fragment lacking an Asp2 transmembrane domain. Assaying to identify inhibitors of Asp1 β-secretase activity also is contemplated.

The β-secretase cleavage site in APP is known, and it will be appreciated that the assays of the invention can be performed with either intact APP or

fragments or analogs of APP that retain the β-secretase recognition and cleavage site. Thus, in one variation, the substrate polypeptide of the second composition comprises the amino acid sequence SEVNLDAEFR, which includes the β-secretase recognition site of human APP that contains the "Swiss" mutation. In another variation, the substrate polypeptide of the second composition comprises the amino acid sequence EVKMDAEF. In another variation, the second composition comprises a polypeptide having an amino acid sequence of a human amyloid precursor protein (APP). For example, the human amyloid precursor protein is selected from the group consisting of: APP695, APP751, and APP770. Preferably, the human amyloid precursor protein (irrespective of isoform selected) includes at least on mutation selected from a KM→NL Swiss mutation and a V→F London mutation. As explained elsewhere, one preferred embodiment involves a variation wherein the polypeptide having an amino acid sequence of a human APP further comprises an amino acid sequence comprising a marker sequence attached amino-terminal to the amino acid sequence of the human amyloid precursor protein. Preferably, the polypeptide having an amino acid sequence of a human APP further comprises two lysine residues attached to the carboxyl terminus of the amino acid sequence of the human APP. The assays can be performed in a cell free setting, using cell-free enzyme and cell-free substrate, or can be performed in a cell-based assay wherein the second composition comprises a eukaryotic cell that expresses amyloid precursor protein (APP) or a fragment thereof containing a β-secretase cleavage site. Preferably, the APP expressed by the host cell is an APP variant that includes two carboxyl-terminal lysine residues. It will also be appreciated that the β-secretase enzyme can be an enzyme that is expressed on the surface of the same cells.

The present invention provides isolated nucleic acid molecules comprising a polynucleotide that codes for a polypeptide selected from the group consisting of human aspartyl proteases. In particular, human aspartyl protease 1 (Hu-Asp1) and two alternative splice variants of human aspartyl protease-2 (Hu-Asp2), a "long" (L) form designated herein as Hu-Asp2(a) and a "short" (S) form designated Hu-Asp2(b). As used herein, all references to "Hu-Asp" should be understood to refer to all of

Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b). In addition, as used herein, all references to "Hu-Asp2" should be understood to refer to both Hu-Asp2(a) and Hu-Asp2(b). Hu-Asp1 is expressed most abundantly in pancreas and prostate tissues, while Hu-Asp2(a) and Hu-Asp2(b) are expressed most abundantly in pancreas and brain tissues. The invention also provides isolated Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides, as well as fragments thereof which exhibit aspartyl protease activity.

In a preferred embodiment, the nucleic acid molecules comprise a polynucleotide having a nucleotide sequence selected from the group consisting of residues 1-1554 of SEQ ID NO. 1, encoding Hu-Asp1, residues 1-1503 of SEQ ID NO. 3, encoding Hu-Asp2(a), and residues 1-1428 of SEQ ID NO.5, encoding Hu-Asp2(b). In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent conditions to a polynucleotide encoding Hu-Asp1, Hu-Asp2(a), Hu-Asp-2(b), or fragments thereof. European patent application EP 0 848 062 discloses a polypeptide referred to as "Asp 1," that bears substantial homology to Hu-Asp1, while international application WO 98/22597 discloses a polypeptide referred to as "Asp 2," that bears substantial homology to Hu-Asp2(a).

The present invention also provides vectors comprising the isolated nucleic acid molecules of the invention, host cells into which such vectors have been introduced, and recombinant methods of obtaining a Hu-Asp1, Hu-Asp2(a), or Hu-Asp2(b) polypeptide comprising culturing the above-described host cell and isolating the relevant polypeptide.

In another aspect, the invention provides isolated Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides, as well as fragments thereof. In a preferred embodiment, the Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides have the amino acid sequence given in SEQ ID NO. 2, SEQ ID NO. 4, or SEQ ID NO.6, respectively. The present invention also describes active forms of Hu-Asp2, methods for preparing such active forms, methods for preparing soluble forms, methods for measuring Hu-Asp2 activity, and substrates for Hu-Asp2 cleavage. The invention also describes antisense oligomers targeting the Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) mRNA transcripts and the use of such antisense reagents to decrease such mRNA and consequently the production of the corresponding polypeptide. Isolated antibodies, both polyclonal and monoclonal, that

binds specifically to any of the Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides of the invention are also provided.

The invention also provides a method for the identification of an agent that modulates the activity of any of Hu-Asp-1, Hu-Asp2(a), and Hu-Asp2(b). The inventions describes methods to test such agents in cell-free assays to which Hu-Asp2 polypeptide is added, as well as methods to test such agents in human or other mammalian cells in which Hu-Asp2 is present.

The invention provides for methods for assaying for human Asp1(hu-Asp1) α-secretase activity comprising contacting the hu-Asp1 protein with an amyloid precursor protein (APP) substrate, wherein the substrate contains an α-secretase cleavage site; and measuring cleavage of the APP substrate at the α-secretase cleavage site, thereby assaying hu-Asp1 α-secretase activity. An example of α-secretase activity is APP processing wherein the APP substrate is cleaved at a site adjacent to the cell membrane (at residues $Phe^{20}{\downarrow}Ala^{21}$ in relation to the Aβ peptide). This cleavage results in the release of a soluble, extracellular domain of APP, known as amyloid alpha peptide (sAPPα), from the cell surface into the cytoplasm. The sAPPα within the cytoplasm can be detected and quantitated thereby measuring α-secretase activity.

The hu-Asp1 enzyme used in the methods of the invention can be purified and isolated from a cell which is transfected or transformed with a polynucleotide that encodes hu-Asp1, such as SEQ ID NO: 1, or a polynucleotide sequence that encodes the the amino acid sequence of SEQ ID NO: 2. Further, the hu-Asp1 protein used in the methods may be a fragment of the amino acid sequence of SEQ ID NO: 2 which retains α-secretase activity. Possible fragments that may be of use for the methods include those lacking the transmembrane domain amino acids 469-492 of SEQ ID NO: 2, those fragments that lack the cytoplasmic amino acids 493-492 of SEQ ID NO: 2, those fragments that lack the amino terminal amino acids 1-62 of SEQ ID NO: 2 or combinations thereof.

The invention also encompasses methods of assaying for α-secretase activity where hu-Asp1 protein and its substrate are brought into contact by a growing cell transfected or transformed with a polynucleotide encoding the hu-Asp1 protein or a

fragment thereof that retains α-secretase activity under conditions where the cell expresses hu-Asp1 protein in the presence of the APP substrate. The APP substrate in such circumstances can be exogenously introduced, or more preferably, is expressed by the cell that expresses Asp1. These methods also encompass contacting hu-Asp1 protein with a cell that expresses a polynucleotide that encodes an APP substrate containing an α-secretase cleavage site. For example, the cell may express a polynucleotide that encodes a polypeptide having an α-secretase cleavage site comprising the amino acid sequence LVFFAEDF or KLVFFAED. In addition, the APP substrate may comprise any human isoform of APP, such as "normal" APP (APP695), APP 751, or APP770. These APP substrates can be further modified to comprise a carboxy-terminal di-lysine motif.

To measure the cleavage of the substrates for the methods of assaying for α-secretase activity of the invention, the substrates of the method can be further modified to comprise detectable labels such as radioactive, enzymatic, chemilumenescent or flourescent labels. In particular, shorter peptide substrates preferably comprise internally quenched labels that result in increased detectability after cleavage of the peptide substrates. The peptide substrates may be modified to have attached a paired flurophore and quencher including but not limited to 7-amino-4-methyl coumarin and dinitrophenol, respectively, such that cleavage of the peptide by the hu-Asp1 results in increased fluorescence due to physical separation of the flurophore and quencher. Other paired flurophores and quenchers include bodipy-tetramethylrhodamine and QSY-5 (Molecular Probes, Inc.) In a variant of this assay, biotin or another suitable tag may be placed on one end of the peptide to anchor the peptide to a substrate assay plate and a flurophore may be placed at the other end of the peptide. Useful flurophores include those listed above as well as Europium labels such as W8044 (EG&G Wallac, Inc.). A preferred label is oregon green that may be attached to a Cys residue. Cleavage of the peptide by Asp1 will release the flurophore or other tag from the plate, allowing compounds to be assayed for inhibition of Asp1 proteolytic cleavage as shown by an increase in retained fluorescence. Preferred colorimetric assays of hu-Asp1 proteolytic activity utilize other suitable substrates that include the P_2 and P_1 amino acids comprising the recognition site for cleavage linked to o-nitrophenol through an amide linkage, such that cleavage by the

hu-Asp1 results in an increase in optical density after altering the assay buffer to alkaline pH.

The prevent invention also provides for methods of assaying for α-secretase activity comprising contacting hu-Asp1 protein with an APP substrate, determining the level of hu-Asp1 α-secretase activity in the presence and absence of a modulator of hu-Asp1 α-secretase activity and comparing the hu-Asp1 secretase activity in the presence and absence of the modulator. The modulators determined to increase hu-Asp1 α-secretase activity will be identified as candidate Alzheimer's disease therapeutics. The invention also encompasses methods which comprise a step for treating Alzheimer's disease with identified candidate Alzheimer disease therapeutics. The invention also provides for compositions comprising a candidate Alzheimer's disease therpeutic identified by the α-secretase assaying methods of the invention. Asp1 modulators that reduce Asp1 β-secretase activity and increase Asp1 α-secretase activity are highly preferred. Assays for Asp1 β-secretase activity are preferred essentially as described in detail herein for Asp2.

The invention provides for Asp1 protease substrate peptides or fragments thereof, wherein said peptides comprise an amino acid sequence consisting of fifty or fewer amino acids which comprise the Asp1 cleavage site having the amino acid sequence GLALALEP. This peptide was derived from the Asp1 amino acid sequence and the discovery of an apparent Asp1 autocatalytic cleavage in acidic conditions. The Asp1 substrate of the invention may also comprise a detectable label, such as a radioactive label, chemiluminescent label, enzymatic label or a flourescent label. The flourescently labeled substrate can consist of internally quenched labels as described above.

The invention also encompasses methods comprising the steps of contacting hu-Asp1 protein with an Asp1 substrate under acidic conditions and determining the level of Asp1 proteolytic activity. An example of Asp1 proteolytic activity is the auto-catalytic processing hu-Asp undergoes in acidic environments, wherein cleavage occurs at an amino acid site surrounding Ala^{63} and cleaves the amino terminal amino acids of the hu-Asp1 pro-peptide. The hu-Asp1 pro-peptide refers to a secreted form of Asp1 that has completed intercellular processing which resulted in cleavage of its signal sequence.

For the methods of assaying Asp1 proteolytic activity, the hu-Asp1 may be produced in a cell transformed or transfected with a polynucleotide that encodes hu-Asp1. The hu-Asp1protein may be isolated and purified from these cells or the method may utilize a cell growing under conditions that it expresses hu-Asp1. The method may also be carried out with a fragment of hu-Asp1 that retains its proteolytic activity. The fragments provided for by the invention include hu-Asp1 polypeptide sequences which lack the amino acids that encode a transmembrane domain such as amino acids 469-492 of SEQ ID NO: 2 or fragments that lacks the cytoplasmic domain such as amino acids 493-518 of SEQ ID NO: 2.

The invention provides for a purified polynucleotide comprising a nucleotide sequence encoding a polypeptide that comprises a fragment of a hu-Asp1 protein, wherein said nucleotide sequence lacks the sequence that encodes amino acids 23-62 of SEQ ID NO: 2, and wherein the polypeptide has hu-Asp1 α-secretase activity. This portion of the Asp1 sequence corresponds to a punitive pro-peptide that is removed, apparently through autocatalysis, under acidic conditions. These polypeptide fragments also include those lacking the amino terminal amino acids 1-62 of SEQ ID NO: 2.

The invention encompasses a purified polynucleotide comprising a nucleotide sequence that hybridizes under stringent conditions to the non-coding strand complementary to SEQ ID NO: 1, wherein the nucleotide sequence encodes a polypeptide having Asp1 proteolytic activity and wherein the polynucleotide lacks nucleotides encoding a transmembrane domain. Further, the invention also provides for a purified polynucleotide sequence comprising a nucleotide sequence that hybridizes under stringent conditions to the non-coding strand complementary to SEQ ID NO: 1, , wherein the nucleotide sequence encodes a polypeptide further lacking a pro-peptide domain corresponding to amino acids 23-62 of SEQ ID NO: 2.

The invention also provides for vectors comprising the hu-Asp1 polynucleotide of the invention and host cells transfected or transformed with these vectors. The invention also encompasses host cells transfected or transformed with the hu-Asp1 polynucleotides of the invention.

Another embodiment of the invention is a purified polypeptide comprising a fragment of a hu-Asp1 protein, wherein said polypeptide lacks the hu-Asp1 transmembrane domain and retains hu-Asp1 α-secretase activity. These polypeptides include fragments of hu-Asp1 having the amino acid sequence set forth in SEQ ID NO: 2, and wherein the polypeptide optionally lacks the transmembrane domain amino acids 469-492 of SEQ ID NO: 2, wherein the polypeptide lacks the cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2 as well. In one variation, the invention provides a polypeptide that lacks amino terminal amino acids 1-62 of SEQ ID NO: 2 but retains Asp1 proteolytic activity. Fragments lacking both the aforementioned amino-terminal and carboxy terminal residues are contemplated.

The invention provides for a polypeptide comprising a fragment of hu-Asp1 having the amino acid sequence set forth in SEQ ID NO: 2 and wherein said polypeptide lacks the amino terminal amino acids 1-62 and retains APP processing activity. For example, referring to the Asp1 sequence in SEQ ID NO: 2, this pre-pro portion would correspond to residues 22 to 62. By performing conventional sequence analysis, the corresponding portions of the Asp1 sequence can also be identified.

Another embodiment of the invention is a polypeptide comprising the amino acid sequence at least 95% identical to a fragment of hu-Asp1 protein, wherein said polypeptide and said fragment lack a transmembrane domain and retain hu-Asp1 α-secretase activity. In addition, the invention embodies a polypeptide comprising an amino acid sequence at least 95% identical to a fragment of hu-Asp1 protein, wherein said polypeptide and said fragment lack the amino terminal amino acids corresponding to the pre-pro portion of hu-Asp1 and retain APP processing activity.

Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the drawing and detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional embodiments that are also intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the

invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are intended as aspects of the invention.

In addition to the foregoing, the invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations specifically mentioned above. Although the applicant(s) invented the full scope of the claims appended hereto, the claims appended hereto are not intended to encompass within their scope the prior art work of others. Therefore, in the event that statutory prior art within the scope of a claim is brought to the attention of the applicants by a Patent Office or other entity or individual, the applicant(s) reserve the right to exercise amendment rights under applicable patent laws to redefine the subject matter of such a claim to specifically exclude such statutory prior art or obvious variations of statutory prior art from the scope of such a claim. Variations of the invention defined by such amended claims also are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

Sequence ID No. 1: Human Asp-1, nucleotide sequence.

Sequence ID No. 2: Human Asp-1, predicted amino acid sequence.

Sequence ID No. 3: Human Asp-2(a), nucleotide sequence.

Sequence ID No. 4: Human Asp-2(a), predicted amino acid sequence. The Asp2(a) amino acid sequence includes a putative signal peptide comprising residues 1 to 21; and a putative pre-propeptide after the signal peptide that extends through residue 45 (as assessed by processing observed of recombinant Asp2(a) in CHO cells), and a putative propeptide that may extend to at least about residue 57, based on the observation of an observed GRR↓GS sequence which has characteristics of a protease recognition sequence. The Asp2(a) further includes a transmembrane domain comprising residues 455-477, a cytoplasmic domain comprising residues 478-501, and a putative alpha-helical spacer region, comprising residues 420-454, believed to be unnecessary for proteolytic activity, between the protease catalytic domain and the transmembrane domain.

Sequence ID No. 5: Human Asp-2(b), nucleotide sequence.

Sequence ID No. 6: Human Asp-2(b), predicted amino acid sequence. The Asp2(b) amino acid sequence includes a putative signal peptide, pre-propeptide, and propeptide as described above for Asp2(a). The Asp2(b) further includes a transmembrane domain comprising residues 430-452, a cytoplasmic domain comprising residues 453-476, and a putative alpha-helical spacer region, comprising residues 395-429, believed to be unnecessary for proteolytic activity, between the protease catalytic domain and the transmembrane domain.

Sequence ID No. 7: Murine Asp-2(a), nucleotide sequence.

Sequence ID No. 8: Murine Asp-2(a), predicted amino acid sequence. The proteolytic processing of murine Asp2(a) is believed to be analogous to the processing described above for human Asp2(a). In addition, a variant lacking amino acid residues 190-214 of SEQ ID NO: 8 is specifically contemplated as a murine Asp2(b) polypeptide.

Sequence ID No. 9: Human APP695, nucleotide sequence.

Sequence ID No.10: Human APP695, predicted amino acid sequence.

Sequence ID No.11: Human APP695-Sw, nucleotide sequence.

Sequence ID No.12: Human APP695-Sw. predicted amino acid sequence. In the APP695 isoform, the Sw mutation is characterized by a KM→NL alteration at positions 595-596 (compared to normal APP695).

Sequence ID No.13: Human APP695-VF, nucleotide sequence.

Sequence ID No.14: Human APP695-VF, predicted amino acid sequence. In the APP 695 isoform, the VF mutation is characterized by a V→F alteration at position 642 (compared to normal APP 695).

Sequence ID No.15: Human APP695-KK, nucleotide sequence.

Sequence ID No.16: Human APP695-KK, predicted amino acid sequence. (APP695 with two carboxy-terminal lysine residues.)

Sequence ID No.17: Human APP695-Sw-KK, nucleotide sequence.

Sequence ID No.18: Human APP695-Sw-KK, predicted amino acid sequence

Sequence ID No.19: Human APP695-VF-KK, nucleotide sequence

Sequence ID No.20: Human APP695-VF-KK, predicted amino acid sequence

Sequence ID No.21: T7-Human-pro-Asp-2(a)ΔTM, nucleotide sequence

Sequence ID No.22: T7-Human-pro-Asp-2(a)ΔTM, amino acid sequence

Sequence ID No.23: T7-Caspase-Human-pro-Asp-2(a)ΔTM, nucleotide sequence

Sequence ID No.24: T7-Caspase-Human-pro-Asp-2(a)ΔTM, amino acid sequence

Sequence ID No.25: Human-pro-Asp-2(a)ΔTM (low GC), nucleotide sequence

Sequence ID No.26: Human-pro-Asp-2(a)ΔTM, (low GC), amino acid sequence

Sequence ID No.27: T7-Caspase-Caspase 8 cleavage-Human-pro-Asp-2(a)ΔTM, nucleotide sequence

Sequence ID No.28: T7-Caspase-Caspase 8 cleavage-Human-pro-Asp-2(a)ΔTM, amino acid sequence

Sequence ID No.29: Human Asp-2(a)ΔTM, nucleotide sequence

Sequence ID No.30: Human Asp-2(a)ΔTM, amino acid sequence

Sequence ID No.31: Human Asp-2(a)ΔTM(His)$_6$, nucleotide sequence

Sequence ID No. 32: Human Asp-2(a)ΔTM(His)$_6$, amino acid sequence

Sequence ID Nos. 33-49 are short synthetic peptide and oligonucleotide sequences that are described below in the Detailed Description of the Invention.

Sequence ID No. 50: Human Asp2(b)ΔTM polynucleotide sequence.

Sequence ID No. 51: Human Asp2(b)ΔTM polypeptide sequence (exemplary variant of Human Asp2(b) lacking transmembrane and intracellular domains of Hu-Asp2(b) set forth in SEQ ID NO: 6.

Sequence ID No. 52: Human Asp2(b)ΔTM(His)$_6$ polynucleotide sequence.

Sequence ID No. 53: Human Asp2(b)ΔTM(His)$_6$ polypeptide sequence (Human Asp2(b)ΔTM with six histidine tag attached to C-terminus).

Sequence ID No. 54: Human APP770-encoding polynucleotide sequence.

Sequence ID No. 55: Human APP770 polypeptide sequence. To introduce the KM→NL Swedish mutation, residues KM at positions 670-71 are changed to NL. To introduce the V→F London mutation, the V residue at position 717 is changed to F.

Sequence ID No. 56: Human APP751 encoding polynucleotide sequence.

Sequence ID No. 57: Human APP751 polypeptide sequence (Human APP751 isoform).

Sequence ID No. 58: Human APP770-KK encoding polynucleotide sequence.

Sequence ID No. 59: Human APP770-KK polypeptide sequence. (Human APP770 isoform to which two C-terminal lysines have been added).

Sequence ID No. 60: Human APP751-KK encoding polynucleotide sequence.

Sequence ID No. 61: Human APP751-KK polypeptide sequence (Human APP751 isoform to which two C-terminal lysines have been added).

Sequence ID Nos. 62-65: Various short peptide sequences described in detail in detailed description.

Sequence ID No. 66: Predicted amino acid sequence of human Asp-1ΔTM$(His)_6$ as described in Example 14.

Sequence ID No. 67: Amino acid sequence of secreted recombinant Asp-1ΔTM(His)6 as described in Example 14.

Sequence ID No. 68: Amino acid sequence of acid-processed form of Asp1Δ$(His)_6$.

Sequence ID No. 69: Amino acid sequence of the self-activated acid processing site within Asp-1ΔTM.

Sequence ID No. 70: Amino acid sequence of a peptide that includes the β-secretase processing site within the Swedish mutant form of APP.

Sequence ID No. 71: Amino acid sequence of a peptide (residues 17-24) that includes the α-secretase processing site within the Aβ peptide ($A\beta_{12-28}$).

Sequence ID No. 72: Amino acid sequence of a peptide (residues 16-23) that includes the α-secretase processing site within the Aβ peptide ($A\beta_{12-28}$).

Sequence ID No. 73-74: PCR primers described in Example 14.

Sequence ID No.75: Amino acid sequence of a γ-secretase substrate polypeptide described in Example 15.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Figure 1 shows the nucleotide (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of human Asp1.

Figure 2: Figure 2 shows the nucleotide (SEQ ID NO:3) and predicted amino acid sequence (SEQ ID NO:4) of human Asp2(a).

Figure 3: Figure 3 shows the nucleotide (SEQ ID NO:5) and predicted amino acid sequence (SEQ ID NO:6) of human Asp2(b). The predicted transmembrane domain of Hu-Asp2(b) is enclosed in brackets.

Figure 4: Figure 4 shows the nucleotide (SEQ ID No. 7) and predicted amino acid sequence (SEQ ID No. 8) of murine Asp2(a)

Figure 5: Figure 5 shows the BestFit alignment of the predicted amino acid sequences of Hu-Asp2(a) and murine Asp2(a)

Figure 6: Figure 6 shows the nucleotide (SEQ ID No. 21) and predicted amino acid sequence (SEQ ID No. 22) of T7-Human-pro-Asp-2(a)ΔTM

Figure 7: Figure 7 shows the nucleotide (SEQ ID No. 23) and predicted amino acid sequence (SEQ ID No. 24) of T7-caspase-Human-pro-Asp-2(a)ΔTM

Figure 8: Figure 8 shows the nucleotide (SEQ ID No. 25) and predicted amino acid sequence (SEQ ID No. 26) of Human-pro-Asp-2(a)ΔTM (low GC)

Figure 9: Western blot showing reduction of CTF99 production by HEK125.3 cells transfected with antisense oligomers targeting the Hu-Asp2 mRNA.

Figure 10: Western blot showing increase in CTF99 production in mouse Neuro-2a cells cotransfected with APP-KK with and without Hu-Asp2 only in those cells cotransfected with Hu-Asp2. A further increase in CTF99 production is seen in cells cotransfected with APP-Sw-KK with and without Hu-Asp2 only in those cells cotransfected with Hu-Asp2

Figure 11: Figure 11 shows the predicted amino acid sequence (SEQ ID No. 30) of Human-Asp2(a)ΔTM

Figure 12: Figure 11 shows the predicted amino acid sequence (SEQ ID No. 30) of Human-Asp2(a)ΔTM(His)$_6$

DETAILED DESCRIPTION OF THE INVENTION

A few definitions used in this invention follow, most definitions to be used are those that would be used by one ordinarily skilled in the art.

The term "β amyloid peptide" means any peptide resulting from beta secretase cleavage of APP. This includes peptides of 39, 40, 41, 42 and 43 amino acids, extending from the β-secretase cleavage site to 39, 40, 41, 42 and 43 amino acids C-terminal to the β-secretase cleavage site. β amyloid peptide also includes sequences 1-6, SEQ ID NOs. 1-6 of US 5,750,349, issued 12 May 1998 (incorporated into this document by reference). A β-secretase cleavage fragment disclosed here is called CTF-99, which extends from β-secretase cleavage site to the carboxy terminus of APP.

When an isoform of APP is discussed then what is meant is any APP polypeptide, including APP variants (including mutations), and APP fragments that exists in humans such as those described in US 5,766,846, col 7, lines 45-67, incorporated into this document by reference.

The term "β-amyloid precursor protein" (APP) as used herein is defined as a polypeptide that is encoded by a gene of the same name localized in humans on the long arm of chromosome 21 and that includes "βAP – here "β-amyloid protein" see above, within its carboxyl third. APP is a glycosylated, single-membrane spanning protein expressed in a wide variety of cells in many mammalian tissues. Examples of specific isotypes of APP which are currently known to exist in humans are the 695 amino acid polypeptide described by Kang et. al. (1987) Nature 325:733-736 which is designated as the "normal" APP (SEQ ID NOs: 9-10); the 751 amino acid polypeptide described by Ponte et al. (1988) Nature 331:525-527 (1988) and Tanzi et al. (1988) Nature 331:528-530 (SEQ ID NOs: 56-57); and the 770-amino acid polypeptide described by Kitaguchi et. al. (1988) Nature 331:530-532 (SEQ ID NOs: 54-55). Examples of specific variants of APP include point mutation which can differ in both position and phenotype (for review of known variant mutation see Hardy (1992)

Nature Genet. 1:233-234). All references cited here incorporated by reference. The term "APP fragments" as used herein refers to fragments of APP other than those which consist solely of βAP or βAP fragments. That is, APP fragments will include amino acid sequences of APP in addition to those which form intact βAP or a fragment of βAP.

When the term "any amino acid" is used, the amino acids referred to are to be selected from the following, three letter and single letter abbreviations - which may also be used, are provided as follows:

Alanine, Ala, A; Arginine, Arg, R; Asparagine, Asn, N; Aspartic acid, Asp, D; Cysteine, Cys, C; Glutamine, Gln, Q; Glutamic Acid, Glu, E; Glycine, Gly, G; Histidine, His, H; Isoleucine, Ile, I; Leucine, Leu, L; Lysine, Lys, K; Methionine, Met, M; Phenylalanine, Phe, F; Proline, Pro, P; Serine, Ser, S; Threonine, Thr, T; Tryptophan, Trp, W; Tyrosine, Tyr, Y; Valine, Val, V; Aspartic acid or Asparagine, Asx, B; Glutamic acid or Glutamine, Glx, Z; Any amino acid, Xaa, X.

The present invention describes a method to scan gene databases for the simple active site motif characteristic of aspartyl proteases. Eukaryotic aspartyl proteases such as pepsin and renin possess a two-domain structure which folds to bring two aspartyl residues into proximity within the active site. These are embedded in the short tripeptide motif DTG, or more rarely, DSG. Most aspartyl proteases occur as proenzyme whose N-terminus must be cleaved for activation. The DTG or DSG active site motif appears at about residue 65-70 in the proenzyme (prorenin, pepsinogen), but at about residue 25-30 in the active enzyme after cleavage of the N-terminal prodomain. The limited length of the active site motif makes it difficult to search collections of short, expressed sequence tags (EST) for novel aspartyl proteases. EST sequences typically average 250 nucleotides or less, and so would encode 80-90 amino acid residues or less. That would be too short a sequence to span the two active site motifs. The preferred method is to scan databases of hypothetical or assembled protein coding sequences. The present invention describes a computer method to identify candidate aspartyl proteases in protein sequence databases. The method was used to identify seven candidate aspartyl protease sequences in the

Caenorhabditis elegans genome. These sequences were then used to identify by homology search Hu-Asp1 and two alternative splice variants of Hu-Asp2, designated herein as Hu-Asp2(a) and Hu-Asp2(b).

In a major aspect of the invention disclosed here we provide new information about APP processing. Pathogeneic processing of the amyloid precursor protein (APP) via the Aβ pathway requires the sequential action of two proteases referred to as β-secretase and γ-secretase. Cleavage of APP by the β-secretase and γ-secretase generates the N-terminus and C-terminus of the Aβ peptide, respectively. Because over production of the Aβ peptide, particularly the $A\beta_{1-42}$, has been implicated in the initiation of Alzheimer's disease, inhibitors of either the β-secretase and/or the γ-secretase have potential in the treatment of Alzheimer's disease. Despite the importance of the β-secretase and γ-secretase in the pathogenic processing of APP, molecular definition of these enzymes has not been accomplished to date. That is, it was not known what enzymes were required for cleavage at either the β-secretase or the γ-secretase cleavage site. The sites themselves were known because APP was known and the $A\beta_{1-42}$, peptide was known, see US 5,766,846 and US 5,837,672, (incorporated by reference, with the exception to reference to "soluble" peptides). But what enzyme was involved in producing the $A\beta_{1-42}$, peptide was unknown.

Alignment of the amino acid sequences of Hu-Asp2 with other known aspartyl proteases reveals a similar domain organization. All of the sequences contain a signal sequence followed by a pro-segment and the catalytic domain containing 2 copies of the aspartyl protease active site motif (DTG/DSG) separated by approximately 180 amino acid residues. Comparison of the processing site for proteolytic removal of the pro-segment in the mature forms of pepsin A, pepsin C, cathepsin D, cathepsin E and renin reveals that the mature forms of these enzymes contain between 31-35 amino acid residues upstream of the first DTG motif. Inspection of this region in the Hu-Asp-2 amino acid sequence indicates a preferred processing site within the sequence GRR↓GS as proteolytic processing of pro-protein precursors commonly occurs at site following dibasic amino acid pairs (*eg.* RR). Also, processing at this site would yield a mature enzyme with 35 amino acid residues upstream of the first

DTG, consistent with the processing sites for other aspartyl proteases. In the absence of self-activation of Hu-Asp2 or a knowledge of the endogenous protease that processes Hu-Asp2 at this site, a recombinant form was engineered by introducing a recognition site for the PreSission protease (LEVLFQ↓GP) into the expression plasmids for bacterial, insect cell, and mammalian cell expression of pro-Hu-Asp2. In each case, the Gly residue in P1' position corresponds to the Gly residue 35 amino acids upstream of the first DTG motif in Hu-Asp2.

The present invention involves the molecular definition of several novel human aspartyl proteases and one of these, referred to as Hu-Asp-2(a) and Hu-Asp2(b), has been characterized in detail. Previous forms of asp1 and asp 2 have been disclosed, see EP 0848062 A2 and EP 0855444A2, inventors David Powel et al., assigned to Smith Kline Beecham Corp. (incorporated by reference). Herein are disclosed old and new forms of Hu-Asp 2. For the first time they are expressed in active form, their substrates are disclosed, and their specificity is disclosed. Prior to this disclosure cell or cell extracts were required to cleave the β-secretase site, now purified protein can be used in assays, also described here. Based on the results of (1) antisense knock out experiments, (2) transient transfection knock in experiments, and (3) biochemical experiments using purified recombinant Hu-Asp-2, we demonstrate that Hu-Asp-2 is the β-secretase involved in the processing of APP. Although the nucleotide and predicted amino acid sequence of Hu-Asp-2(a) has been reported, see above, see EP 0848062 A2 and EP 0855444A2, no functional characterization of the enzyme was disclosed. Here the authors characterize the Hu-Asp-2 enzyme and are able to explain why it is a critical and essential enzyme required in the formation of $A\beta_{1-42}$, peptide and possible a critical step in the development of AD.

In another embodiment the present invention also describes a novel splice variant of Hu-Asp2, referred to as Hu-Asp-2(b), that has never before been disclosed.

In another embodiment, the invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a polypeptide selected from the group consisting of human aspartyl protease 1 (Hu-Asp1) and two alternative splice variants of human aspartyl protease-2 (Hu-Asp2), designated herein as Hu-Asp2(a) and

Hu-Asp2(b). As used herein, all references to "Hu-Asp2" should be understood to refer to both Hu-Asp2(a) and Hu-Asp2(b). Hu-Asp1 is expressed most abundantly in pancreas and prostate tissues, while Hu-Asp2(a) and Hu-Asp2(b) are expressed most abundantly in pancreas and brain tissues. The invention also provides isolated Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides, as well as fragments thereof which exhibit aspartyl protease activity.

The predicted amino acid sequences of Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) share significant homology with previously identified mammalian aspartyl proteases such as pepsinogen A, pepsinogen B, cathepsin D, cathepsin E, and renin. P.B.Szecs, *Scand. J. Clin. Lab. Invest. 52*:(Suppl. 210 5-22 (1992)). These enzymes are characterized by the presence of a duplicated DTG/DSG sequence motif. The Hu-Asp1 and HuAsp2 polypeptides disclosed herein also exhibit extremely high homology with the ProSite consensus motif for aspartyl proteases extracted from the SwissProt database.

The nucleotide sequence given as residues 1-1554 of SEQ ID NO:1 corresponds to the nucleotide sequence encoding Hu-Asp1, the nucleotide sequence given as residues 1-1503 of SEQ ID NO:3 corresponds to the nucleotide sequence encoding Hu-Asp2(a), and the nucleotide sequence given as residues 1-1428 of SEQ ID NO:5 corresponds to the nucleotide sequence encoding Hu-Asp2(b). The isolation and sequencing of DNA encoding Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) is described below in Examples 1 and 2.

As is described in Examples 1 and 2, automated sequencing methods were used to obtain the nucleotide sequence of Hu-Asp1, Hu-Asp2(a), and Hu-Asp-2(b). The Hu-Asp nucleotide sequences of the present invention were obtained for both DNA strands, and are believed to be 100% accurate. However, as is known in the art, nucleotide sequence obtained by such automated methods may contain some errors. Nucleotide sequences determined by automation are typically at least about 90%, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of a given nucleic acid molecule. The actual sequence may be more precisely determined using manual sequencing methods, which are well known

in the art. An error in sequence which results in an insertion or deletion of one or more nucleotides may result in a frame shift in translation such that the predicted amino acid sequence will differ from that which would be predicted from the actual nucleotide sequence of the nucleic acid molecule, starting at the point of the mutation. The Hu-Asp DNA of the present invention includes cDNA, chemically synthesized DNA, DNA isolated by PCR, genomic DNA, and combinations thereof. Genomic Hu-Asp DNA may be obtained by screening a genomic library with the Hu-Asp2 cDNA described herein, using methods that are well known in the art, or with oligonucleotides chosen from the Hu-Asp2 sequence that will prime the polymerase chain reaction (PCR). RNA transcribed from Hu-Asp DNA is also encompassed by the present invention.

Due to the degeneracy of the genetic code, two DNA sequences may differ and yet encode identical amino acid sequences. The present invention thus provides isolated nucleic acid molecules having a polynucleotide sequence encoding any of the Hu-Asp polypeptides of the invention, wherein said polynucleotide sequence encodes a Hu-Asp polypeptide having the complete amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or fragments thereof.

Also provided herein are purified Hu-Asp polypeptides, both recombinant and non-recombinant. Most importantly, methods to produce Hu-Asp2 polypeptides in active form are provided. These include production of Hu-Asp2 polypeptides and variants thereof in bacterial cells, insect cells, and mammalian cells, also in forms that allow secretion of the Hu-Asp2 polypeptide from bacterial, insect or mammalian cells into the culture medium, also methods to produce variants of Hu-Asp2 polypeptide incorporating amino acid tags that facilitate subsequent purification. In a preferred embodiment of the invention the Hu-Asp2 polypeptide is converted to a proteolytically active form either in transformed cells or after purification and cleavage by a second protease in a cell-free system, such active forms of the Hu-Asp2 polypeptide beginning with the N-terminal sequence TQHGIR or ETDEEP. The sequence TQHGIR represents the amino-terminus of Asp2(a) or Asp2(b) beginning with residue 22 of SEQ ID NO: 4 or 6, after cleavage of a putative 21 residue signal

peptide. Recombinant Asp2(a) expressed in and purified from insect cells was observed to have this amino terminus, presumably as a result of cleavage by a signal peptidase. The sequence ETDEEP represents the amino-terminus of Asp2(a) or Asp2(b) beginning with residue 46 of SEQ ID NO: 4 or 6, as observed when Asp2(a) has been recombinantly produced in CHO cells (presumably after cleavage by both a rodent signal peptidase and another rodent peptidase that removes a propeptide sequence). The Asp2(a) produced in the CHO cells possesses β-secretase activity, as described in greater detail in Examples 11 and 12. Variants and derivatives, including fragments, of Hu-Asp proteins having the native amino acid sequences given in SEQ ID Nos: 2, 4, and 6 that retain any of the biological activities of Hu-Asp are also within the scope of the present invention. Of course, one of ordinary skill in the art will readily be able to determine whether a variant, derivative, or fragment of a Hu-Asp protein displays Hu-Asp activity by subjecting the variant, derivative, or fragment to a standard aspartyl protease assay. Fragments of Hu-Asp within the scope of this invention include those that contain the active site domain containing the amino acid sequence DTG, fragments that contain the active site domain amino acid sequence DSG, fragments containing both the DTG and DSG active site sequences, fragments in which the spacing of the DTG and DSG active site sequences has been lengthened, fragments in which the spacing has been shortened. Also within the scope of the invention are fragments of Hu-Asp in which the transmembrane domain has been removed to allow production of Hu-Asp2 in a soluble form. In another embodiment of the invention, the two halves of Hu-Asp2, each containing a single active site DTG or DSG sequence can be produced independently as recombinant polypeptides, then combined in solution where they reconstitute an active protease.

Thus, the invention provides a purified polypeptide comprising a fragment of a mammalian Asp2 protein, wherein said fragment lacks the Asp2 transmembrane domain of said Asp2 protein, and wherein the polypeptide and the fragment retain the β-secretase activity of said mammalian Asp2 protein. In a preferred embodiment, the purified polypeptide comprises a fragment of a human Asp2 protein that retains the β-

secretase activity of the human Asp2 protein from which it was derived. Examples include:

a purified polypeptide that comprises a fragment of Asp2(a) having the amino acid sequence set forth in SEQ ID NO: 4, wherein the polypeptide lacks transmembrane domain amino acids 455 to 477 of SEQ ID NO: 4;

a purified polypeptide as described in the preceding paragraph that further lacks cytoplasmic domain amino acids 478 to 501 of SEQ ID NO: 4;

a purified polypeptide as described in either of the preceding paragraphs that further lacks amino acids 420-454 of SEQ ID NO: 4, which constitute a putative alpha helical region between the catalytic domain and the transmembrane domain that is believed to be unnecessary for β-secretase activity;

a purified polypeptide that comprises an amino acid sequence that includes amino acids 58 to 419 of SEQ ID NO: 4, and that lacks amino acids 22 to 57 of SEQ ID NO: 4;

a purified polypeptide that comprises an amino acid sequence that includes amino acids 46 to 419 of SEQ ID NO: 4, and that lacks amino acids 22 to 45 of SEQ ID NO: 4;

a purified polypeptide that comprises an amino acid sequence that includes amino acids 22 to 454 of SEQ ID NO: 4.

a purified polypeptide that comprises a fragment of Asp2(b) having the amino acid sequence set forth in SEQ ID NO: 6, and wherein said polypeptide lacks transmembrane domain amino acids 430 to 452 of SEQ ID NO: 6;

a purified polypeptide as described in the preceding paragraph that further lacks cytoplasmic domain amino acids 453 to 476 of SEQ ID NO: 6;

a purified polypeptide as described in either of the preceding two paragraphs that further lacks amino acids 395-429 of SEQ ID NO: 4, which constitute a putative alpha helical region between the catalytic domain and the transmembrane domain that is believed to be unnecessary for β-secretase activity;

a purified polypeptide comprising an amino acid sequence that includes amino acids 58 to 394 of SEQ ID NO: 4, and that lacks amino acids 22 to 57 of SEQ ID NO: 4;

a purified polypeptide comprising an amino acid sequence that includes amino acids 46 to 394 of SEQ ID NO: 4, and that lacks amino acids 22 to 45 of SEQ ID NO: 4; and

a purified polypeptide comprising an amino acid sequence that includes amino acids 22 to 429 of SEQ ID NO: 4.

Also included as part of the invention is a purified polynucleotide comprising a nucleotide sequence that encodes such polypeptides; a vector comprising a polynucleotide that encodes such polypeptides; and a host cell transformed or transfected with such a polynucleotide or vector.

Hu-Asp variants may be obtained by mutation of native Hu-Asp-encoding nucleotide sequences, for example. A Hu-Asp variant, as referred to herein, is a polypeptide substantially homologous to a native Hu-Asp polypeptide but which has an amino acid sequence different from that of native Hu-Asp because of one or more deletions, insertions, or substitutions in the amino acid sequence. The variant amino acid or nucleotide sequence is preferably at least about 80% identical, more preferably at least about 90% identical, and most preferably at least about 95% identical, to a native Hu-Asp sequence. Thus, a variant nucleotide sequence which contains, for example, 5 point mutations for every one hundred nucleotides, as compared to a native Hu-Asp gene, will be 95% identical to the native protein. The percentage of sequence identity, also termed homology, between a native and a variant Hu-Asp sequence may also be determined, for example, by comparing the two sequences using any of the computer programs commonly employed for this purpose, such as the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wisconsin), which uses the algorithm of Smith and Waterman (*Adv. Appl. Math. 2:* 482-489 (1981)).

Alterations of the native amino acid sequence may be accomplished by any of a number of known techniques. For example, mutations may be introduced at

particular locations by procedures well known to the skilled artisan, such as oligonucleotide-directed mutagenesis, which is described by Walder *et al.* (*Gene 42*:133 (1986)); Bauer *et al.* (*Gene 37*:73 (1985)); Craik (*BioTechniques*, January 1985, pp. 12-19); Smith *et al.* (*Genetic Engineering: Principles and Methods*, Plenum Press (1981)); and U.S. Patent Nos. 4,518,584 and 4,737,462.

Hu-Asp variants within the scope of the invention may comprise conservatively substituted sequences, meaning that one or more amino acid residues of a Hu-Asp polypeptide are replaced by different residues that do not alter the secondary and/or tertiary structure of the Hu-Asp polypeptide. Such substitutions may include the replacement of an amino acid by a residue having similar physicochemical properties, such as substituting one aliphatic residue (Ile, Val, Leu or Ala) for another, or substitution between basic residues Lys and Arg, acidic residues Glu and Asp, amide residues Gln and Asn, hydroxyl residues Ser and Tyr, or aromatic residues Phe and Tyr. Further information regarding making phenotypically silent amino acid exchanges may be found in Bowie *et al., Science 247*:1306-1310 (1990). Other Hu-Asp variants which might retain substantially the biological activities of Hu-Asp are those where amino acid substitutions have been made in areas outside functional regions of the protein.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent conditions to a portion of the nucleic acid molecules described above, *e.g.*, to at least about 15 nucleotides, preferably to at least about 20 nucleotides, more preferably to at least about 30 nucleotides, and still more preferably to at least about from 30 to at least about 100 nucleotides, of one of the previously described nucleic acid molecules. Such portions of nucleic acid molecules having the described lengths refer to, *e.g.*, at least about 15 contiguous nucleotides of the reference nucleic acid molecule. By stringent hybridization conditions is intended overnight incubation at about 42°C for about 2.5 hours in 6 X SSC/0.1% SDS, followed by washing of the filters four times for 15 minutes in 1.0 X SSC at 65°C, 0.1% SDS.

Fragments of the Hu-Asp encoding nucleic acid molecules described herein, as well as polynucleotides capable of hybridizing to such nucleic acid molecules may be used as a probe or as primers in a polymerase chain reaction (PCR). Such probes may be used, *e.g.*, to detect the presence of Hu-Asp nucleic acids in *in vitro* assays, as well as in Southern and northern blots. Cell types expressing Hu-Asp may also be identified by the use of such probes. Such procedures are well known, and the skilled artisan will be able to choose a probe of a length suitable to the particular application. For PCR, 5' and 3' primers corresponding to the termini of a desired Hu-Asp nucleic acid molecule are employed to isolate and amplify that sequence using conventional techniques.

Other useful fragments of the Hu-Asp nucleic acid molecules are antisense or sense oligonucleotides comprising a single stranded nucleic acid sequence capable of binding to a target Hu-Asp mRNA (using a sense strand), or Hu-Asp DNA (using an antisense strand) sequence. In a preferred embodiment of the invention these Hu-Asp antisense oligonucleotides reduce Hu-Asp mRNA and consequent production of Hu-Asp polypeptides.

In another aspect, the invention includes Hu-Asp polypeptides with or without associated native pattern glycosylation. Both Hu-Asp1 and Hu-Asp2 have canonical acceptor sites for Asn-linked sugars, with Hu-Asp1 having two of such sites, and Hu-Asp2 having four. Hu-Asp expressed in yeast or mammalian expression systems (discussed below) may be similar to or significantly different from a native Hu-Asp polypeptide in molecular weight and glycosylation pattern. Expression of Hu-Asp in bacterial expression systems will provide non-glycosylated Hu-Asp.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. Hu-Asp polypeptides may be recovered and purified from tissues, cultured cells, or recombinant cell cultures by well-known methods, including ammonium sulfate or ethanol precipitation, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, lectin chromatography, and high performance liquid chromatography

(HPLC). In a preferred embodiment, an amino acid tag is added to the Hu-Asp polypeptide using genetic engineering techniques that are well known to practitioners of the art which include addition of six histidine amino acid residues to allow purification by binding to nickel immobilized on a suitable support, epitopes for polyclonal or monoclonal antibodies including but not limited to the T7 epitope, the myc epitope, and the V5a epitope, and fusion of Hu-Asp2 to suitable protein partners including but not limited to glutathione-S-transferase or maltose binding protein. In a preferred embodiment these additional amino acid sequences are added to the C-terminus of Hu-Asp but may be added to the N-terminus or at intervening positions within the Hu-Asp2 polypeptide.

The present invention also relates to vectors comprising the polynucleotide molecules of the invention, as well as host cell transformed with such vectors. Any of the polynucleotide molecules of the invention may be joined to a vector, which generally includes a selectable marker and an origin of replication, for propagation in a host. Because the invention also provides Hu-Asp polypeptides expressed from the polynucleotide molecules described above, vectors for the expression of Hu-Asp are preferred. The vectors include DNA encoding any of the Hu-Asp polypeptides described above or below, operably linked to suitable transcriptional or translational regulatory sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, mRNA ribosomal binding sites, and appropriate sequences which control transcription and translation. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA encoding Hu-Asp. Thus, a promoter nucleotide sequence is operably linked to a Hu-Asp DNA sequence if the promoter nucleotide sequence directs the transcription of the Hu-Asp sequence.

Selection of suitable vectors to be used for the cloning of polynucleotide molecules encoding Hu-Asp, or for the expression of Hu-Asp polypeptides, will of course depend upon the host cell in which the vector will be transformed, and, where applicable, the host cell from which the Hu-Asp polypeptide is to be expressed.

Suitable host cells for expression of Hu-Asp polypeptides include prokaryotes, yeast, and higher eukaryotic cells, each of which is discussed below.

The Hu-Asp polypeptides to be expressed in such host cells may also be fusion proteins which include regions from heterologous proteins. Such regions may be included to allow, *e.g.*, secretion, improved stability, or facilitated purification of the polypeptide. For example, a sequence encoding an appropriate signal peptide can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused inframe to the Hu-Asp sequence so that Hu-Asp is translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cell promotes extracellular secretion of the Hu-Asp polypeptide. Preferably, the signal sequence will be cleaved from the Hu-Asp polypeptide upon secretion of Hu-Asp from the cell. Nonlimiting examples of signal sequences that can be used in practicing the invention include the yeast Ifactor and the honeybee melatin leader in sf9 insect cells.

In a preferred embodiment, the Hu-Asp polypeptide will be a fusion protein which includes a heterologous region used to facilitate purification of the polypeptide. Many of the available peptides used for such a function allow selective binding of the fusion protein to a binding partner. For example, the Hu-Asp polypeptide may be modified to comprise a peptide to form a fusion protein which specifically binds to a binding partner, or peptide tag. Nonlimiting examples of such peptide tags include the 6-His tag, thioredoxin tag, hemaglutinin tag, GST tag, and OmpA signal sequence tag. As will be understood by one of skill in the art, the binding partner which recognizes and binds to the peptide may be any molecule or compound including metal ions *(e.g.*, metal affinity columns), antibodies, or fragments thereof, and any protein or peptide which binds the peptide, such as the FLAG tag.

Suitable host cells for expression of Hu-Asp polypeptides includes prokaryotes, yeast, and higher eukaryotic cells. Suitable prokaryotic hosts to be used for the expression of Hu-Asp include bacteria of the genera *Escherichia, Bacillus, and Salmonella,* as well as members of the genera *Pseudomonas, Streptomyces,* and *Staphylococcus.* For expression in, *e.g., E. coli*, a Hu-Asp polypeptide may include

an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in a prokaryotic host. The N-terminal Met may optionally then be cleaved from the expressed Hu-Asp polypeptide. Other N-terminal amino acid residues can be added to the Hu-Asp polypeptide to facilitate expression in Escherichia coli including but not limited to the T7 leader sequence, the T7-caspase 8 leader sequence, as well as others leaders including tags for purification such as the 6-His tag (Example 9). Hu-Asp polypeptides expressed in E. coli may be shortened by removal of the cytoplasmic tail, the transmembrane domain, or the membrane proximal region. Hu-Asp polypeptides expressed in E. coli may be obtained in either a soluble form or as an insoluble form which may or may not be present as an inclusion body. The insoluble polypeptide may be rendered soluble by guanidine HCl, urea or other protein denaturants, then refolded into a soluble form before or after purification by dilution or dialysis into a suitable aqueous buffer. If the inactive proform of the Hu-Asp was produced using recombinant methods, it may be rendered active by cleaving off the prosegment with a second suitable protease such as human immunodeficiency virus protease.

Expression vectors for use in prokaryotic hosts generally comprises one or more phenotypic selectable marker genes. Such genes generally encode, *e.g.*, a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial sources. Examples include pSPORT vectors, pGEM vectors (Promega), pPROEX vectors (LTI, Bethesda, MD), Bluescript vectors (Stratagene), pET vectors (Novagen) and pQE vectors (Qiagen).

Hu-Asp may also be expressed in yeast host cells from genera including *Saccharomyces, Pichia,* and *Kluveromyces*. Preferred yeast hosts are *S. cerevisiae* and *P. pastoris.* Yeast vectors will often contain an origin of replication sequence from a 2T yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Vectors replicable in both yeast and *E. coli* (termed shuttle vectors) may also be used. In addition to the above-mentioned features of yeast

vectors, a shuttle vector will also include sequences for replication and selection in *E. coli*. Direct secretion of Hu-Asp polypeptides expressed in yeast hosts may be accomplished by the inclusion of nucleotide sequence encoding the yeast I-factor leader sequence at the 5' end of the Hu-Asp-encoding nucleotide sequence.

Insect host cell culture systems may also be used for the expression of Hu-Asp polypeptides. In a preferred embodiment, the Hu-Asp polypeptides of the invention are expressed using an insect cell expression system (*see* Example 10). Additionally, a baculovirus expression system can be used for expression in insect cells as reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988).

In another preferred embodiment, the Hu-Asp polypeptide is expressed in mammalian host cells. Nonlimiting examples of suitable mammalian cell lines include the COS7 line of monkey kidney cells (Gluzman *et al., Cell* 23:175 (1981)), human embyonic kidney cell line 293, and Chinese hamster ovary (CHO) cells. Preferably, Chinese hamster ovary (CHO) cells are used for expression of Hu-Asp proteins (Example 11).

The choice of a suitable expression vector for expression of the Hu-Asp polypeptides of the invention will of course depend upon the specific mammalian host cell to be used, and is within the skill of the ordinary artisan. Examples of suitable expression vectors include pcDNA3 (Invitrogen) and pSVL (Pharmacia Biotech). A preferred vector for expression of Hu-Asp polypeptides is pcDNA3.1-Hygro (Invitrogen). Expression vectors for use in mammalian host cells may include transcriptional and translational control sequences derived from viral genomes. Commonly used promoter sequences and enhancer sequences which may be used in the present invention include, but are not limited to, those derived from human cytomegalovirus (CMV), Adenovirus 2, Polyoma virus, and Simian virus 40 (SV40). Methods for the construction of mammalian expression vectors are disclosed, for example, in Okayama and Berg (*Mol. Cell. Biol. 3*:280 (1983)); Cosman *et al.* (*Mol. Immunol. 23*:935 (1986)); Cosman *et al.* (*Nature 312*:768 (1984)); EP-A-0367566; and WO 91/18982.

The polypeptides of the present invention may also be used to raise polyclonal and monoclonal antibodies, which are useful in diagnostic assays for detecting Hu-Asp polypeptide expression. Such antibodies may be prepared by conventional techniques. See, for example, *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988); *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet *et al.* (eds.), Plenum Press, New York (1980). Synthetic peptides comprising portions of Hu-Asp containing 5 to 20 amino acids may also be used for the production of polyclonal or monoclonal antibodies after linkage to a suitable carrier protein including but not limited to keyhole limpet hemacyanin (KLH), chicken ovalbumin, or bovine serum albumin using various cross-linking reagents including carbodimides, glutaraldehyde, or if the peptide contains a cysteine, N-methylmaleimide. A preferred peptide for immunization when conjugated to KLH contains the C-terminus of Hu-Asp1 or Hu-Asp2 comprising QRRPRDPEVVNDESSLVRHRWK (SEQ ID NO: 2, residues 497-518) or LRQQHDDFADDISLLK (SEQ ID NO:4, residues 486-501), respectively. See SEQ ID Nos. 33-34.

The Hu-Asp nucleic acid molecules of the present invention are also valuable for chromosome identification, as they can hybridize with a specific location on a human chromosome. Hu-Asp1 has been localized to chromosome 21, while Hu-Asp2 has been localized to chromosome 11q23.3-24.1. There is a current need for identifying particular sites on the chromosome, as few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. The relationship between genes and diseases that have been mapped to the same chromosomal region can then be identified through linkage analysis, wherein the coinheritance of physically adjacent genes is determined. Whether a gene appearing to be related to a particular disease is in fact the cause of

the disease can then be determined by comparing the nucleic acid sequence between affected and unaffected individuals.

In another embodiment, the invention relates to a method of assaying Hu-Asp function, specifically Hu-Asp2 function which involves incubating in solution the Hu-Asp polypeptide with a suitable substrate including but not limited to a synthetic peptide containing the β-secretase cleavage site of APP, preferably one containing the mutation found in a Swedish kindred with inherited AD in which KM is changed to NL, such peptide comprising the sequence SEVNLDAEFR in an acidic buffering solution, preferably an acidic buffering solution of pH5.5 (see Example 12) using cleavage of the peptide monitored by high performance liquid chromatography as a measure of Hu-Asp proteolytic activity. Preferred assays for proteolytic activity utilize internally quenched peptide assay substrates. Such suitable substrates include peptides which have attached a paired flurophore and quencher including but not limited to 7-amino-4-methyl coumarin and dinitrophenol, respectively, such that cleavage of the peptide by the Hu-Asp results in increased fluorescence due to physical separation of the flurophore and quencher. Other paired flurophores and quenchers include bodipy-tetramethylrhodamine and QSY-5 (Molecular Probes, Inc.). In a variant of this assay, biotin or another suitable tag may be placed on one end of the peptide to anchor the peptide to a substrate assay plate and a flurophore may be placed at the other end of the peptide. Useful flurophores include those listed above as well as Europium labels such as W8044 (EG&g Wallac, Inc.). Cleavage of the peptide by Asp2 will release the flurophore or other tag from the plate, allowing compounds to be assayed for inhibition of Asp2 proteolytic cleavage as shown by an increase in retained fluorescence. Preferred colorimetric assays of Hu-Asp proteolytic activity utilize other suitable substrates that include the P2 and P1 amino acids comprising the recognition site for cleavage linked to o-nitrophenol through an amide linkage, such that cleavage by the Hu-Asp results in an increase in optical density after altering the assay buffer to alkaline pH.

In another embodiment, the invention relates to a method for the identification of an agent that increases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method comprising

(a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

(b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent;

whereby a higher level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has increased the activity of said Hu-Asp polypeptide. Such tests can be performed with Hu-Asp polypeptide in a cell free system and with cultured cells that express Hu-Asp as well as variants or isoforms thereof.

In another embodiment, the invention relates to a method for the identification of an agent that decreases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method comprising

(a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

(b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent; whereby a lower level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has decreased the activity of said Hu-Asp polypeptide. Such tests can be performed with Hu-Asp polypeptide in a cell free system and with cultured cells that express Hu-Asp as well as variants or isoforms thereof.

In another embodiment, the invention relates to a novel cell line (HEK125.3 cells) for measuring processing of amyloid β peptide (Aβ) from the amyloid protein precursor (APP). The cells are stable transformants of human embryonic kidney 293 cells (HEK293) with a bicistronic vector derived from pIRES-EGFP (Clontech) containing a modified human APP cDNA, an internal ribosome entry site and an

enhanced green fluorescent protein (EGFP) cDNA in the second cistron. The APP cDNA was modified by adding two lysine codons to the carboxyl terminus of the APP coding sequence. This increases processing of Aβ peptide from human APP by 2-4 fold. This level of Aβ peptide processing is 60 fold higher than is seen in nontransformed HEK293 cells. HEK125.3 cells will be useful for assays of compounds that inhibit Aβ peptide processing. This invention also includes addition of two lysine residues to the C-terminus of other APP isoforms including the 751 and 770 amino acid isoforms, to isoforms of APP having mutations found in human AD including the Swedish KM→NL and V717→F mutations, to C-terminal fragments of APP, such as those beginning with the β-secretase cleavage site, to C-terminal fragments of APP containing the β-secretase cleavage site which have been operably linked to an N-terminal signal peptide for membrane insertion and secretion, and to C-terminal fragments of APP which have been operably linked to an N-terminal signal peptide for membrane insertion and secretion and a reporter sequence including but not limited to green fluorescent protein or alkaline phosphatase, such that β-secretase cleavage releases the reporter protein from the surface of cells expressing the polypeptide.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Example 1

Development of a Search Algorithm Useful for the Identification of Aspartyl Proteases, and Identification of C. elegans Aspartyl Protease Genes in Wormpep 12

Materials and Methods:

Classical aspartyl proteases such as pepsin and renin possess a two-domain structure which folds to bring two aspartyl residues into proximity within the active site. These are embedded in the short tripeptide motif DTG, or more rarely, DSG. The DTG or DSG active site motif appears at about residue 25-30 in the enzyme, but at about 65-70 in the proenzyme (prorenin, pepsinogen). This motif appears again about

150-200 residues downstream. The proenzyme is activated by cleavage of the N-terminal prodomain. This pattern exemplifies the double domain structure of the modern day aspartyl enzymes which apparently arose by gene duplication and divergence. Thus;

NH_2------------X------D^{25}TG---------------Y-------D^{Y+25}TG----------------C

where X denotes the beginning of the enzyme, following the N-terminal prodomain, and Y denotes the center of the molecule where the gene repeat begins again.

In the case of the retroviral enzymes such as the HIV protease, they represent only a half of the two-domain structures of well-known enzymes like pepsin, cathepsin D, renin, etc. They have no prosegment, but are carved out of a polyprotein precursor containing the *gag* and *pol* proteins of the virus. They can be represented by:

NH_2---------D^{25}TG---------------------C100

This "monomer" only has about 100 aa, so is extremely parsimonious as compared to the other aspartyl protease "dimers" which have of the order of 330 or so aa, not counting the N-terminal prodomain.

The limited length of the eukaryotic aspartyl protease active site motif makes it difficult to search EST collections for novel sequences. EST sequences typically average 250 nucleotides, and so in this case would be unlikely to span both aspartyl protease active site motifs. Instead, we turned to the *C. elegans* genome. The *C. elegans* genome is estimated to contain around 13,000 genes. Of these, roughly 12,000 have been sequenced and the corresponding hypothetical open reading frame (ORF) has been placed in the database Wormpep12. We used this database as the basis for a whole genome scan of a higher eukaryote for novel aspartyl proteases, using an algorithm that we developed specifically for this purpose. The following AWK script for locating proteins containing two DTG or DSG motifs was used for the search, which was repeated four times to recover all pairwise combinations of the aspartyl motif.

```
BEGIN{RS=">"}               /* defines ">" as record separator for FASTA format */
{
pos = index($0,"DTG")       /*finds "DTG" in record*/
```

```
if (pos>0) {
        rest = substr($0,pos+3)        /*get rest of record after first DTG*/
        pos2 = index(rest,"DTG")       /*find second DTG*/
        if (pos2>0) printf ("%s%s\n",">",$0)}        /*report hits*/
}
}
```

The AWK script shown above was used to search Wormpep12, which was downloaded from ftp.sanger.ac.uk/pub/databases/wormpep, for sequence entries containing at least two DTG or DSG motifs. Using AWK limited each record to 3000 characters or less. Thus, 35 or so larger records were eliminated manually from Wormpep12 as in any case these were unlikely to encode aspartyl proteases.

Results and Discussion:

The Wormpep 12 database contains 12,178 entries, although some of these (<10%) represent alternatively spliced transcripts from the same gene. Estimates of the number of genes encoded in the *C. elegans* genome is on the order of 13,000 genes, so Wormpep12 may be estimated to cover greater than 90% of the *C. elegans* genome.

Eukaryotic aspartyl proteases contain a two-domain structure, probably arising from ancestral gene duplication. Each domain contains the active site motif D(S/T)G located from 20-25 amino acid residues into each domain. The retroviral (e.g., HIV protease) or retrotransposon proteases are homodimers of subunits which are homologous to a single eukaryotic aspartyl protease domain. An AWK script was used to search the Wormpep12 database for proteins in which the D(S/T)G motif occurred at least twice. This identified >60 proteins with two DTG or DSG motifs. Visual inspection was used to select proteins in which the position of the aspartyl domains was suggestive of a two-domain structure meeting the criteria described above.

In addition, the PROSITE eukaryotic and viral aspartyl protease active site pattern PS00141 was used to search Wormpep12 for candidate aspartyl proteases. (Bairoch A., Bucher P., Hofmann K., The PROSITE database: its status in 1997, *Nucleic Acids Res. 24*:217-221(1997)). This generated an overlapping set of Wormpep12 sequences. Of these, seven sequences contained two DTG or DSG

motifs and the PROSITE aspartyl protease active site pattern. Of these seven, three were found in the same cosmid clone (F21F8.3, F21F8.4, and F21F8.7) suggesting that they represent a family of proteins that arose by ancestral gene duplication. Two other ORFs with extensive homology to F21F8.3, F21F8.4 and F21F8.7 are present in the same gene cluster (F21F8.2 and F21F8.6), however, these contain only a single DTG motif. Exhaustive BLAST searches with these seven sequences against Wormpep12 failed to reveal additional candidate aspartyl proteases in the *C. elegans* genome containing two repeats of the DTG or DSG motif.

BLASTX search with each *C. elegans* sequence against SWISS-PROT, GenPep and TREMBL revealed that R12H7.2 was the closest worm homologue to the known mammalian aspartyl proteases, and that T18H9.2 was somewhat more distantly related, while CEASP1, F21F8.3, F21F8.4, and F21F8.7 formed a subcluster which had the least sequence homology to the mammalian sequences.

Discussion:

APP, the presenilins, and p35, the activator of cdk5, all undergo intracellular proteolytic processing at sites which conform to the substrate specificity of the HIV protease. Dysregulation of a cellular aspartyl protease with the same substrate specificity, might therefore provide a unifying mechanism for causation of the plaque and tangle pathologies in AD. Therefore, we sought to identify novel human aspartyl proteases. A whole genome scan in *C. elegans* identified seven open reading frames that adhere to the aspartyl protease profile that we had identified. These seven aspartyl proteases probably comprise the complete complement of such proteases in a simple, multicellular eukaryote. These include four closely related aspartyl proteases unique to *C. elegans* which probably arose by duplication of an ancestral gene. The other three candidate aspartyl proteases (T18H9.2, R12H7.2 and C11D2.2) were found to have homology to mammalian gene sequences.

Example 2

Identification of Novel Human Aspartyl Proteases Using Database Mining by Genome Bridging

Materials and Methods:

Computer-assisted analysis of EST databases, cDNA , and predicted polypeptide sequences:

Exhaustive homology searches of EST databases with the CEASP1, F21F8.3, F21F8.4, and F21F8.7 sequences failed to reveal any novel mammalian homologues. TBLASTN searches with R12H7.2 showed homology to cathepsin D, cathepsin E, pepsinogen A, pepsinogen C and renin, particularly around the DTG motif within the active site, but also failed to identify any additional novel mammalian aspartyl proteases. This indicates that the *C. elegans* genome probably contains only a single lysosomal aspartyl protease which in mammals is represented by a gene family that arose through duplication and consequent modification of an ancestral gene.

TBLASTN searches with T18H9.2, the remaining *C. elegans* sequence, identified several ESTs which assembled into a contig encoding a novel human aspartyl protease (Hu-ASP1). As is described above in Example 1, BLASTX search with the Hu-ASP1 contig against SWISS-PROT revealed that the active site motifs in the sequence aligned with the active sites of other aspartyl proteases. Exhaustive, repetitive rounds of BLASTN searches against LifeSeq, LifeSeqFL, and the public EST collections identified 102 EST from multiple cDNA libraries that assembled into a single contig. The 51 sequences in this contig found in public EST collections also have been assembled into a single contig (THC213329) by The Institute for Genome Research (TIGR). The TIGR annotation indicates that they failed to find any hits in the database for the contig. Note that the TIGR contig is the reverse complement of the LifeSeq contig that we assembled. BLASTN search of Hu-ASP1 against the rat and mouse EST sequences in ZooSeq revealed one homologous EST in each database (Incyte clone 700311523 and IMAGE clone 313341, GenBank accession number W10530, respectively).

TBLASTN searches with the assembled DNA sequence for Hu-ASP1 against both LifeSeqFL and the public EST databases identified a second, related human sequence (Hu-Asp2) represented by a single EST (2696295). Translation of this partial cDNA sequence reveals a single DTG motif which has homology to the active site motif of a bovine aspartyl protease, NM1.

BLAST searches, contig assemblies and multiple sequence alignments were performed using the bioinformatics tools provided with the LifeSeq, LifeSeqFL and LifeSeq Assembled databases from Incyte. Predicted protein motifs were identified using either the ProSite dictionary (Motifs in GCG 9) or the Pfam database.

Full-length cDNA cloning of Hu-Asp1

The open reading frame of *C. elegans* gene T18H9.2CE was used to query Incyte LifeSeq and LifeSeq-FL databases and a single electronic assembly referred to as 1863920CE1 was detected. The 5' most cDNA clone in this contig, 1863920, was obtained from Incyte and completely sequenced on both strands. Translation of the open reading frame contained within clone 1863920 revealed the presence of the duplicated aspartyl protease active site motif (DTG/DSG) but the 5' end was incomplete. The remainder of the Hu-Asp1 coding sequence was determined by 5' Marathon RACE analysis using a human placenta Marathon ready cDNA template (Clontech). A 3'-antisense oligonucleotide primer specific for the 5' end of clone 1863920 was paired with the 5'-sense primer specific for the Marathon ready cDNA synthetic adaptor in the PCR. Specific PCR products were directly sequenced by cycle sequencing and the resulting sequence assembled with the sequence of clone 1863920 to yield the complete coding sequence of Hu-Asp-1 (SEQ ID No. 1).

Several interesting features are present in the primary amino acid sequence of Hu-Asp1 (Figure 1, SEQ ID No. 2). The sequence contains a signal peptide (residues 1-20 in SEQ ID No. 2), a pro-segment, and a catalytic domain containing two copies of the aspartyl protease active site motif (DTG/DSG). The spacing between the first and second active site motifs is about 200 residues which should correspond to the expected size of a single, eukaryotic aspartyl protease domain. More interestingly, the sequence contains a predicted transmembrane domain (residues 469-492 in SEQ ID

No.2) near its C-terminus which suggests that the protease is anchored in the membrane. This feature is not found in any other aspartyl protease.

Cloning of a full-length Hu-Asp-2 cDNAs:

As is described above in Example 1, genome wide scan of the *Caenorhabditis elegans* database WormPep12 for putative aspartyl proteases and subsequent mining of human EST databases revealed a human ortholog to the *C. elegans* gene T18H9.2 referred to as Hu-Asp1. The assembled contig for Hu-Asp1 was used to query for human paralogs using the BLAST search tool in human EST databases and a single significant match (2696295CE1) with approximately 60% shared identity was found in the LifeSeq FL database. Similar queries of either gb105PubEST or the family of human databases available from TIGR did not identify similar EST clones. cDNA clone 2696295, identified by single pass sequence analysis from a human uterus cDNA library, was obtained from Incyte and completely sequence on both strands. This clone contained an incomplete 1266 bp open-reading frame that encoded a 422 amino acid polypeptide but lacked an initiator ATG on the 5' end. Inspection of the predicted sequence revealed the presence of the duplicated aspartyl protease active site motif DTG/DSG, separated by 194 amino acid residues. Subsequent queries of later releases of the LifeSeq EST database identified an additional ESTs, sequenced from a human astrocyte cDNA library (4386993), that appeared to contain additional 5' sequence relative to clone 2696295. Clone 4386993 was obtained from Incyte and completely sequenced on both strands. Comparative analysis of clone 4386993 and clone 2696295 confirmed that clone 4386993 extended the open-reading frame by 31 amino acid residues including two in-frame translation initiation codons. Despite the presence of the two in-frame ATGs, no in-frame stop codon was observed upstream of the ATG indicating that the 4386993 may not be full-length. Furthermore, alignment of the sequences of clones 2696295 and 4386993 revealed a 75 base pair insertion in clone 2696295 relative to clone 4386993 that results in the insertion of 25 additional amino acid residues in 2696295. The remainder of the Hu-Asp2 coding sequence was determined by 5' Marathon RACE analysis using a human

hippocampus Marathon ready cDNA template (Clontech). A 3'-antisense oligonucleotide primer specific for the shared 5'-region of clones 2696295 and 4386993 was paired with the 5'-sense primer specific for the Marathon ready cDNA synthetic adaptor in the PCR. Specific PCR products were directly sequenced by cycle sequencing and the resulting sequence assembled with the sequence of clones 2696295 and 4386993 to yield the complete coding sequence of Hu-Asp2(a) (SEQ ID No. 3) and Hu-Asp2(b) (SEQ ID No. 5), respectively.

Several interesting features are present in the primary amino acid sequence of Hu-Asp2(a) (Figure 2 and SEQ ID No. 4) and Hu-Asp-2(b) (Figure 3, SEQ ID No. 6). Both sequences contain a signal peptide (residues 1-21 in SEQ ID No. 4 and SEQ ID No. 6), a pro-segment, and a catalytic domain containing two copies of the aspartyl protease active site motif (DTG/DSG). The spacing between the first and second active site motifs is variable due to the 25 amino acid residue deletion in Hu-Asp-2(b) and consists of 168-*versus*-194 amino acid residues, for Hu-Asp2(b) and Hu-Asp-2(a), respectively. More interestingly, both sequences contains a predicted transmembrane domain (residues 455-477 in SEQ ID No.4 and 430-452 in SEQ ID No. 6) near their C-termini which indicates that the protease is anchored in the membrane. This feature is not found in any other aspartyl protease except Hu-Asp1.

Example 3

Molecular cloning of mouse Asp2 cDNA and genomic DNA.

Cloning and characterization of murine Asp2 cDNA.

The murine ortholog of Hu-Asp2 was cloned using a combination of cDNA library screening, PCR, and genomic cloning. Approximately 500,000 independent clones from a mouse brain cDNA library were screened using a ^{32}P-labeled coding sequence probe prepared from Hu-Asp2. Replicate positives were subjected to DNA sequence analysis and the longest cDNA contained the entire 3' untranslated region and 47 amino acids in the coding region. PCR amplification of the same mouse brain cDNA library with an antisense oligonucleotide primer specific for the 5'–most cDNA sequence determined above and a sense primer specific for the 5' region of

human Asp2 sequence followed by DNA sequence analysis gave an additional 980 bp of the coding sequence. The remainder of the 5' sequence of murine Asp-2 was derived from genomic sequence (see below).

Isolation and sequence analysis of the murine Asp-2 gene.

A murine EST sequence encoding a portion of the murine Asp2 cDNA was identified in the GenBank EST database using the BLAST search tool and the Hu-Asp2 coding sequence as the query. Clone g3160898 displayed 88% shared identity to the human sequence over 352 bp. Oligonucleotide primer pairs specific for this region of murine Asp2 were then synthesized and used to amplify regions of the murine gene. Murine genomic DNA, derived from strain 129/SvJ, was amplified in the PCR (25 cycles) using various primer sets specific for murine Asp2 and the products analyzed by agarose gel electrophoresis. The primer set Zoo-1 and Zoo-4 amplified a 750 bp fragment that contained approximately 600 bp of intron sequence based on comparison to the known cDNA sequence. This primer set was then used to screen a murine BAC library by PCR, a single genomic clone was isolated and this cloned was confirmed contain the murine Asp2 gene by DNA sequence analysis. Shotgun DNA sequencing of this Asp2 genomic clone and comparison to the cDNA sequences of both Hu-Asp2 and the partial murine cDNA sequences defined the full-length sequence of murine Asp2 (SEQ ID No. 7). The predicted amino acid sequence of murine Asp2 (SEQ ID No. 8) showed 96.4% shared identity (GCG BestFit algorithm) with 18/501 amino acid residue substitutions compared to the human sequence (Figure 4). The proteolytic processing of murine Asp2(a) is believed to be analogous to the processing described above for human Asp2(a). In addition, a variant lacking amino acid residues 190-214 of SEQ ID NO: 8 is specifically contemplated as a murine Asp2(b) polypeptide. All forms of murine Asp2(b) gene and protein are intended as aspects of the invention.

Example 4

Tissue Distribution of Expression of Hu-Asp2 Transcripts

Materials and Methods:

The tissue distribution of expression of Hu-Asp-2 was determined using multiple tissue Northern blots obtained from Clontech (Palo Alto, CA). Incyte clone 2696295 in the vector pINCY was digested to completion with *EcoRI/NotI* and the 1.8 kb cDNA insert purified by preparative agarose gel electrophoresis. This fragment was radiolabeled to a specific activity > 1 X 10^9 dpm/μg by random priming in the presence of [α-^{32}P-dATP] (>3000 Ci/mmol, Amersham, Arlington Heights, IL) and Klenow fragment of DNA polymerase I. Nylon filters containing denatured, size fractionated poly A^+ RNAs isolated from different human tissues were hybridized with 2 x 10^6 dpm/ml probe in ExpressHyb buffer (Clontech, Palo Alto, CA) for 1 hour at 68 °C and washed as recommended by the manufacture. Hybridization signals were visualized by autoradiography using BioMax XR film (Kodak, Rochester, NY) with intensifying screens at -80 °C.

Results and Discussion:

Limited information on the tissue distribution of expression of Hu-Asp-2 transcripts was obtained from database analysis due to the relatively small number of ESTs detected using the methods described above (< 5). In an effort to gain further information on the expression of the Hu-Asp2 gene, Northern analysis was employed to determine both the size(s) and abundance of Hu-Asp2 transcripts. PolyA$^+$ RNAs isolated from a series of peripheral tissues and brain regions were displayed on a solid support following separation under denaturing conditions and Hu-Asp2 transcripts were visualized by high stringency hybridization to radiolabeled insert from clone 2696295. The 2696295 cDNA probe visualized a constellation of transcripts that migrated with apparent sizes of 3.0kb, 4.4 kb and 8.0 kb with the latter two transcript being the most abundant.

Across the tissues surveyed, Hu-Asp2 transcripts were most abundant in pancreas and brain with lower but detectable levels observed in all other tissues

examined except thymus and PBLs. Given the relative abundance of Hu-Asp2 transcripts in brain, the regional expression in brain regions was also established. A similar constellation of transcript sizes were detected in all brain regions examined [cerebellum, cerebral cortex, occipital pole, frontal lobe, temporal lobe and putamen] with the highest abundance in the medulla and spinal cord.

Example 5

Northern Blot Detection of HuAsp-1 and HuAsp-2 Transcripts in Human Cell Lines

A variety of human cell lines were tested for their ability to produce Hu-Asp1 and Asp2 mRNA. Human embryonic kidney (HEK-293) cells, African green monkey (Cos-7) cells, Chinese hamster ovary (CHO) cells, HELA cells, and the neuroblastoma cell line IMR-32 were all obtained from the ATCC. Cells were cultured in DME containing 10% FCS except CHO cells which were maintained in α-MEM/10% FCS at 37 °C in 5% CO_2 until they were near confluence. Washed monolayers of cells (3 X 10^7) were lysed on the dishes and poly A^+ RNA extracted using the Qiagen Oligotex Direct mRNA kit. Samples containing 2 μg of poly A^+ RNA from each cell line were fractionated under denaturing conditions (glyoxal-treated), transferred to a solid nylon membrane support by capillary action, and transcripts visualized by hybridization with random-primed labeled (^{32}P) coding sequence probes derived from either Hu-Asp1 or Hu-Asp2. Radioactive signals were detected by exposure to X-ray film and by image analysis with a PhosphorImager.

The Hu-Asp1 cDNA probe visualized a similar constellation of transcripts (2.6 kb and 3.5 kb) that were previously detected is human tissues. The relative abundance determined by quantification of the radioactive signal was Cos-7 > HEK 292 = HELA > IMR32.

The Hu-Asp2 cDNA probe also visualized a similar constellation of transcripts compared to tissue (3.0 kb, 4.4 kb, and 8.0 kb) with the following relative abundance; HEK 293 > Cos 7 > IMR32 > HELA.

Example 6

Modification of APP to increase Aβ processing for in vitro screening

Human cell lines that process Aβ peptide from APP provide a means to screen in cellular assays for inhibitors of β- and γ-secretase. Production and release of Aβ peptide into the culture supernatant is monitored by an enzyme-linked immunosorbent assay (EIA). Although expression of APP is widespread and both neural and non-neuronal cell lines process and release Aβ peptide, levels of endogenous APP processing are low and difficult to detect by EIA. Aβ processing can be increased by expressing in transformed cell lines mutations of APP that enhance Aβ processing. We made the serendipitous observation that addition of two lysine residues to the carboxyl terminus of APP695 increases Aβ processing still further. This allowed us to create a transformed cell line that releases Aβ peptide into the culture medium at the remarkable level of 20,000 pg/ml.

Materials And Methods

Materials:

Human embryonic kidney cell line 293 (HEK293 cells) were obtained internally. The vector pIRES-EGFP was purchased from Clontech. Oligonucleotides for mutation using the polymerase chain reaction (PCR) were purchased from Genosys. A plasmid containing human APP695 (SEQ ID No. 9 [nucleotide] and SEQ ID No. 10 [amino acid]) was obtained from Northwestern University Medical School. This was subcloned into pSK (Stratagene) at the *Not*1 site creating the plasmid pAPP695.

Mutagenesis protocol:

The Swedish mutation (K670N, M671L) was introduced into pAPP695 using the Stratagene Quick Change Mutagenesis Kit to create the plasmid pAPP695NL (SEQ ID No. 11 [nucleotide] and SEQ ID No. 12 [amino acid]). To introduce a di-lysine motif at the C-terminus of APP695, the forward primer #276 5' GACTGACCACTCGACCAGGTTC (SEQ ID No. 47) was used with the "patch" primer #274 5'

CGAATTAAATTCCAGCACACTGGCTACTTCTTGTTCTGCATCTCAAAGAAC (SEQ ID No. 48) and the flanking primer #275 CGAATTAAATTCCAGCACACTGGCTA (SEQ ID No. 49) to modify the 3' end of the APP695 cDNA (SEQ ID No. 15 [nucleotide] and SEQ ID No. 16 [amino acid]). This also added a BstX1 restriction site that will be compatible with the BstX1 site in the multiple cloning site of pIRES-EGFP. PCR amplification was performed with a Clontech HF Advantage cDNA PCR kit using the polymerase mix and buffers supplied by the manufacturer. For "patch" PCR, the patch primer was used at 1/20th the molar concentration of the flanking primers. PCR amplification products were purified using a QIAquick PCR purification kit (Qiagen). After digestion with restriction enzymes, products were separated on 0.8% agarose gels and then excised DNA fragments were purified using a QIAquick gel extraction kit (Qiagen).

To reassemble a modified APP695-Sw cDNA, the 5' Not1-Bgl2 fragment of the APP695-Sw cDNA and the 3' Bgl2-BstX1 APP695 cDNA fragment obtained by PCR were ligated into pIRES-EGFP plasmid DNA opened at the Not1 and BstX1 sites. Ligations were performed for 5 minutes at room temperature using a Rapid DNA Ligation kit (Boehringer Mannheim) and transformed into Library Efficiency DH5a Competent Cells (GibcoBRL Life Technologies). Bacterial colonies were screened for inserts by PCR amplification using primers #276 and #275. Plasmid DNA was purified for mammalian cell transfection using a QIAprep Spin Miniprep kit (Qiagen). The construct obtained was designated pMG125.3 (APPSW-KK, SEQ ID No. 17 [nucleotide] and SEQ ID No. 18 [amino acid]).

Mammalian Cell Transfection:

HEK293 cells for transfection were grown to 80% confluence in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum. Cotransfections were performed using LipofectAmine (Gibco-BRL) with 3 μg pMG125.3 DNA and 9 μg pcDNA3.1 DNA per 10 x 10^6 cells. Three days posttransfection, cells were passaged into medium containing G418 at a concentration of 400 μg/ml. After three days growth in selective medium, cells were sorted by their fluorescence.

Clonal Selection of 125.3 cells by FACS:

Cell samples were analyzed on an EPICS Elite ESP flow cytometer (Coulter, Hialeah, FL) equipped with a 488 nm excitation line supplied by an air-cooled argon laser. EGFP emission was measured through a 525 nm band-pass filter and fluorescence intensity was displayed on a 4-decade log scale after gating on viable cells as determined by forward and right angle light scatter. Single green cells were separated into each well of one 96 well plate containing growth medium without G418. After a four day recovery period, G418 was added to the medium to a final concentration of 400 μg/ml. After selection, 32% of the wells contained expanding clones. Wells with clones were expanded from the 96 well plate to a 24 well plate and then a 6 well plate with the fastest growing colonies chosen for expansion at each passage. The final cell line selected was the fastest growing of the final six passaged. This clone, designated 125.3, has been maintained in G418 at 400 ug/ml with passage every four days into fresh medium. No loss of Aβ production of EGFP fluorescence has been seen over 23 passages.

Aβ EIA Analysis (Double Antibody Sandwich ELISA for hAβ 1-40/42):

Cell culture supernatants harvested 48 hours after transfection were analyzed in a standard Aβ EIA as follows. Human Aβ 1-40 or 1-42 was measured using monoclonal antibody (mAb) 6E10 (Senetek, St. Louis, MO) and biotinylated rabbit antiserum 162 or 164 (New York State Institute for Basic Research, Staten Island, NY) in a double antibody sandwich ELISA. The capture antibody 6E10 is specific to an epitope present on the N-terminal amino acid residues 1-16 of hAβ. The conjugated detecting antibodies 162 and 164 are specific for hAβ 1-40 and 1-42, respectively. Briefly, a Nunc Maxisorp 96 well immunoplate was coated with 100 μl/well of mAb 6E10 (5μg/ml) diluted in 0.1M carbonate-bicarbonate buffer, pH 9.6 and incubated at 4°C overnight. After washing the plate 3x with 0.01M DPBS (Modified Dulbecco's Phosphate Buffered Saline (0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01 M potassium chloride, pH 7.4) from Pierce, Rockford, Il) containing 0.05% of Tween-20 (DPBST), the plate was blocked for 60 minutes with 200 μl of 10% normal sheep serum (Sigma) in

0.01M DPBS to avoid non-specific binding. Human Aβ 1-40 or 1-42 standards 100 μl/well (Bachem, Torrance, CA) diluted, from a 1mg/ml stock solution in DMSO, in culture medium was added after washing the plate, as well as 100 μl/well of sample, *e.g.*, conditioned medium of transfected cells.

The plate was incubated for 2 hours at room temperature and 4°C overnight. The next day, after washing the plate, 100 μl/well biotinylated rabbit antiserum 162 1:400 or 164 1:50 diluted in DPBST + 0.5% BSA was added and incubated at room temperature for 1hour, 15 minutes. Following washes, 100 μl/well neutravidin-horseradish peroxidase (Pierce, Rockford, Il) diluted 1:10,000 in DPBST was applied and incubated for 1 hour at room temperature. After the last washes 100 μl/well of o-phenylnediamine dihydrochloride (Sigma Chemicals, St. Louis, MO) in 50mM citric acid/100mM sodium phosphate buffer (Sigma Chemicals, St. Louis, MO), pH 5.0, was added as substrate and the color development was monitored at 450nm in a kinetic microplate reader for 20 minutes using Soft max Pro software. All standards and samples were run in triplicates. The samples with absorbance values falling within the standard curve were extrapolated from the standard curves using Soft max Pro software and expressed in pg/ml culture medium.

Results:

Addition of two lysine residues to the carboxyl terminus of APP695 greatly increases Aβ processing in HEK293 cells as shown by transient expression (Table 1). Addition of the di-lysine motif to APP695 increases Aβ processing to that seen with the APP695 containing the Swedish mutation. Combining the di-lysine motif with the Swedish mutation further increases processing by an additional 2.8 fold.

Cotransformation of HEK293 cells with pMG125.3 and pcDNA3.1 allowed dual selection of transformed cells for G418 resistance and high level expression of EGFP. After clonal selection by FACS, the cell line obtained, produces a remarkable 20,000 pg Aβ peptide per ml of culture medium after growth for 36 hours in 24 well plates. Production of Aβ peptide under various growth conditions is summarized in Table 2.

TABLE 1

Release of Aβ peptide into the culture medium 48 hours after transient transfection of HEK293 cells with the indicated vectors containing wildtype or modified APP. Values tabulated are mean + SD and P-value for pairwise comparison using Student's t-test assuming unequal variances.

APP Construct	Aβ 1-40 peptide (pg/ml)	Fold Increase	P-value
pIRES-EGFP vector	147 + 28	1.0	
wt APP695 (142.3)	194 + 15	1.3	0.051
wt APP695-KK (124.1)	424 + 34	2.8	3 x 10-5
APP695-Sw (143.3)	457 + 65	3.1	2 x 10-3
APP695-SwKK (125.3)	1308 + 98	8.9	3 x 10-4

TABLE 2

Release of Aβ peptide from HEK125.3 cells under various growth conditions.

Type of Culture Plate	Volume of Medium	Duration of Culture	Aβ 1-40 (pg/ml)	Aβ 1-42 (pg/ml)
24 well plate	400 ul	36 hr	28,036	1,439

Example 7

Antisense oligomer inhibition of Abeta processing in HEK125.3 cells

The sequences of Hu-Asp1 and Hu-Asp2 were provided to Sequitur, Inc (Natick, MA) for selection of targeted sequences and design of 2nd generation chimeric antisense oligomers using prorietary technology (Sequitur Ver. D Pat pending #3002). Antisense oligomers Lot# S644, S645, S646 and S647 were targeted against Asp1. Antisense oligomers Lot# S648, S649, S650 and S651 were targeted against Asp2. Control antisense oligomers Lot# S652, S653, S655, and S674 were targeted against an irrelevant gene and antisense oligomers Lot #S656, S657, S658, and S659 were targeted against a second irrelevant gene.

For transfection with the antisense oligomers, HEK125.3 cells were grown to about 50% confluence in 6 well plates in Minimal Essential Medium (MEM) supplemented with 10% fetal calf serum. A stock solution of oligofectin G (Sequitur Inc., Natick, MA) at 2 mg/ml was diluted to 50 μg/ml in serum free MEM. Separately, the antisense oligomer stock solution at 100 μM was diluted to 800 nM in Opti-MEM (GIBCO-BRL, Grand Island, NY). The diluted stocks of oligofectin G and antisense oligomer were then mixed at a ratio of 1:1 and incubated at room temperature. After 15 minutes incubation, the reagent was diluted 10 fold into MEM containing 10% fetal calf serum and 2 ml was added to each well of the 6 well plate

after first removing the old medium. After transfection, cells were grown in the continual presence of the oligofectin G/antisense oligomer. To monitor Aβ peptide release, 400 μl of conditioned medium was removed periodically from the culture well and replaced with fresh medium beginning 24 hours after transfection. Aβ peptides in the conditioned medium were assayed via immunoprecipitation and Western blotting. Data reported are from culture supernatants harvested 48 hours after transfection.

The 16 different antisense oligomers obtained from Sequitur Inc. were transfected separately into HEK125.3 cells to determine their affect on Aβ peptide processing. Only antisense oligomers targeted against Asp2 significantly reduced Abeta processing by HEK125.3 cells. Both Aβ (1-40) and Aβ (1-42) were inhibited by the same degree. In Table 3, percent inhibition is calculated with respect to untransfected cells. Antisense oligomer reagents giving greater than 50% inhibition are marked with an asterisk. Of the reagents tested, 3 or 4 antisense oligomers targeted against Asp1 gave an average 52% inhibition of Aβ(1-40) processing and 47% inhibition of Aβ(1-42) processing. For Asp2, 4 of 4 antisense oligomers gave greater than 50% inhibition with an average inhibition of 62% of Aβ(1-40) processing and 60% for Aβ(1-42) processing.

TABLE 3

Inhibition of Aβ peptide release from HEK125.3 cells treated with antisense oligomers.

Gene Targeted	Antisense Oligomer	Abeta (1-40)	Abeta (1-42)
Asp1-1	S644	62%*	56%*
Asp1-2	S645	41%*	38%*
Asp1-3	S646	52%*	46%*
Asp1-4	S647	6%	25%*
Asp2-1	S648	71%*	67%*
Asp2-2	S649	83%*	76%*
Asp2-3	S650	46%*	50%*
Asp2-4	S651	47%*	46%*
Con1-1	S652	13%	18%
Con1-2	S653	35%	30%
Con1-3	S655	9%	18%
Con1-4	S674	29%	18%
Con2-1	S656	12%	18%
Con2-2	S657	16%	19%
Con2-3	S658	8%	35%
Con2-4	S659	3%	18%

Since HEK293 cells derive from kidney, the experiment was extended to human IMR-32 neuroblastoma cells which express all three APP isoforms and which release Aβ peptides into conditioned medium at measurable levels. [See Neill *et al.*, *J. NeuroSci. Res.*, (1994) *39*: 482-93; and Asami-Odaka *et al., Biochem.*, (1995) *34*:10272-8.] Essentially identical results were obtained in the neuroblastoma cells as the HEK293 cells. As shown in Table 3B, the pair of Asp2 antisense oligomers

reduced Asp2 mRNA by roughly one-half, while the pair of reverse control oligomers lacked this effect (Table 3B).

Table 3B

Reduction of Aβ40 and Aβ42 in human neuroblastoma IMR-32 cells and mouse neuroblastoma Neuro-2A cells treated with Asp2 antisense and control oligomers as indicated. Oligomers were transfected in quadruplicate cultures. Values tabulated are normalized against cultures treated with oligofectin-G™ only (mean + SD, ** p<0.001 compared to reverse control oligomer).

		IMR-32 cells		Neuro-2A cells	
	Asp2 mRNA	Aβ40	Aβ42	Aβ40	Aβ42
Asp2-1A	-75%	-49 + 2%**	-42 + 14%**	-70 + 7%**	-67 + 2%**
Asp2-1R	0.16	-0 + 3%	21.26	-9 + 15%	1.05
Asp2-2A	-39%	-43 + 3%**	-44 +18%**	-61 +12%**	-61 +12%**
Asp2-2R	0.47	12.2	19.22	6.15	-8 + 10%

Together with the reduction in Asp2 mRNA there was a concomitant reduction in the release of Aβ40 and Aβ42 peptides into the conditioned medium. Thus, Asp2 functions directly or indirectly in a human kidney and a human neuroblastoma cell line to facilitate the processing of APP into Aβ peptides. Molecular cloning of the mouse Asp2 cDNA revealed a high degree of homology to human (>96% amino acid identity, see Example 3), and indeed, complete nucleotide identity at the sites targeted by the Asp2-1A and Asp2-2A antisense oligomers. Similar results were obtained in mouse Neuro-2a cells engineered to express APP-Sw-KK. The Asp2 antisense oligomers reduced release of Aβ peptides into the medium while the reverse control oligomers did not (Table 3B). Thus, the three antisense experiments with HEK293,

IMR-32 and Neuro-2a cells indicate that Asp2 acts directly or indirectly to facilitate Aβ processing in both somatic and neural cell lines.

Example 8

Demonstration of Hu-Asp2 β-Secretase Activity in Cultured Cells

Several mutations in APP associated with early onset Alzheimer's disease have been shown to alter Aβ peptide processing. These flank the – and C-terminal cleavage sites that release Aβ from APP. These cleavage sites are referred to as the β-secretase and γ-secretase cleavage sites, respectively. Cleavage of APP at the β-secretase site creates a C-terminal fragment of APP containing 99 amino acids of 11,145 daltons molecular weight. The Swedish KM→NL mutation immediately upstream of the β-secretase cleavage site causes a general increase in production of both the 1-40 and 1-42 amino acid forms of Aβ peptide. The London VF mutation (V717→F in the APP770 isoform) has little effect on total Aβ peptide production, but appears to preferentially increase the percentage of the longer 1-42 amino acid form of Aβ peptide by affecting the choice of β-secretase cleavage site used during APP processing. Thus, we sought to determine if these mutations altered the amount and type of Aβ peptide produced by cultured cells cotransfected with a construct directing expression of Hu-Asp2.

Two experiments were performed which demonstrate Hu-Asp2 β-secretase activity in cultured cells. In the first experiment, treatment of HEK125.3 cells with antisense oligomers directed against Hu-Asp2 transcripts as described in Example 7 was found to decrease the amount of the C-terminal fragment of APP created by β-secretase cleavage (CTF99) (Figure 9). This shows that Hu-Asp2 acts directly or indirectly to facilitate β-secretase cleavage. In the second experiment, increased expression of Hu-Asp2 in transfected mouse Neuro2A cells is shown to increase accumulation of the CTF99 β-secretase cleavage fragment (Figure 10). This increase is seen most easily when a mutant APP-KK clone containing a C-terminal di-lysine motif is used for transfection. A further increase is seen when Hu-Asp2 is

cotransfected with APP-Sw-KK containing the Swedish mutation KM →NL. The Swedish mutation is known to increase cleavage of APP by the β-secretase.

A second set of experiments demonstrate Hu-Asp2 facilitates γ-secretase activity in cotransfection experiments with human embryonic kidney HEK293 cells. Cotransfection of Hu-Asp2 with an APP-KK clone greatly increases production and release of soluble Aβ1-40 and Aβ1-42 peptides from HEK293 cells. There is a proportionately greater increase in the release of Aβ1-42. A further increase in production of Aβ1-42 is seen when Hu-Asp2 is cotransfected with APP-VF (SEQ ID No. 13 [nucleotide] and SEQ ID No. 14 [amino acid]) or APP-VF-KK SEQ ID No. 19 [nucleotide] and SEQ ID No. 20 [amino acid]) clones containing the London mutation V717→F. The V717→F mutation is known to alter cleavage specificity of the APP γ-secretase such that the preference for cleavage at the Aβ42 site is increased. Thus, Asp2 acts directly or indirectly to facilitate γ-secretase processing of APP at the β42 cleavage site.

Materials

Antibodies 6E10 and 4G8 were purchased from Senetek (St. Louis, MO). Antibody 369 was obtained from the laboratory of Paul Greengard at the Rockefeller University. Antibody C8 was obtained from the laboratory of Dennis Selkoe at the Harvard Medical School and Brigham and Women's Hospital.

APP Constructs used

The APP constructs used for transfection experiments comprised the following

APP: wild-type APP695 (SEQ ID No. 9 and No. 10)

APP-Sw: APP695 containing the Swedish KM→NL mutation (SEQ ID No. 11 and No. 12 , wherein the lysine (K) at residue 595 of APP695 is changed to asparagine (N) and the methionine (M) at residue 596 of APP695 is changed to leucine (L).),

APP-VF: APP695 containing the London V→F mutation (SEQ ID Nos. 13 & 14) (Affected residue 717 of the APP770 isoform corresponds with residue 642 of the APP695 isoform. Thus, APP-VF as set in SEQ ID NO: 14 comprises the APP695 sequence, wherein the valine (V) at residue 642 is changed to phenylalanine (F).)

APP-KK: APP695 containing a C-terminal KK motif (SEQ ID Nos. 15 & 16),

APP-Sw-KK: APP695-Sw containing a C-terminal KK motif (SEQ ID No. 17 & 18),

APP-VF-KK: APP695-VF containing a C-terminal KK motif (SEQ ID Nos. 19 & 20).

These were inserted into the vector pIRES-EGFP (Clontech, Palo Alto CA) between the *Not*1 and *BstX*1 sites using appropriate linker sequences introduced by PCR.

Transfection of antisense oligomers or plasmid DNA constructs in HEK293 cells, HEK125.3 cells and Neuro-2A cells,

Human embryonic kidney HEK293 cells and mouse Neuro-2a cells were transfected with expression constructs using the Lipofectamine Plus reagent from Gibco/BRL. Cells were seeded in 24 well tissue culture plates to a density of 70-80% confluence. Four wells per plate were transfected with 2 μg DNA (3:1, APP:cotransfectant), 8 μl Plus reagent, and 4 μl Lipofectamine in OptiMEM. OptiMEM was added to a total volume of 1 ml, distributed 200 μl per well and incubated 3 hours. Care was taken to hold constant the ratios of the two plasmids used for cotransfection as well as the total amount of DNA used in the transfection. The transfection media was replaced with DMEM, 10%FBS, NaPyruvate, with antibiotic/antimycotic and the cells were incubated under normal conditions (37°C, 5% CO_2) for 48 hours. The conditioned media were removed to polypropylene tubes and stored at –80°C until assayed for the content of Aβ1-40 and Aβ1-42 by EIA as described in the preceding examples. Transfection of antisense oligomers into HEK125.3 cells was as described in Example 7.

Preparation of cell extracts, Western blot protocol

Cells were harvested after being transfected with plasmid DNA for about 60 hours. First, cells were transferred to 15-ml conical tube from the plate and centrifuged at 1,500 rpm for 5 minutes to remove the medium. The cell pellets were washed once with PBS. We then lysed the cells with lysis buffer (10 mM HEPES, pH

7.9, 150 mM NaCl, 10% glycerol, 1 mM EGTA, 1 mM EDTA, 0.1 mM sodium vanadate and 1% NP-40). The lysed cell mixtures were centrifuged at 5000 rpm and the supernatant was stored at -20°C as the cell extracts. Equal amounts of extracts from HEK125.3 cells transfected with the Asp2 antisense oligomers and controls were precipitated with antibody 369 that recognizes the C-terminus of APP and then CTF99 was detected in the immunoprecipitate with antibody 6E10. The experiment was repeated using C8, a second precipitating antibody that also recognizes the C-terminus of APP. For Western blot of extracts from mouse Neuro-2a cells cotransfected with Hu-Asp2 and APP-KK, APP-Sw-KK, APP-VF-KK or APP-VF, equal amounts of cell extracts were electrophoresed through 4-10% or 10-20% Tricine gradient gels (NOVEX, San Diego, CA). Full length APP and the CTF99 β-secretase product were detected with antibody 6E10.

Results

Transfection of HEK125.3 cells with Asp2-1 or Asp2-2 antisense oligomers reduces production of the CTF β-secretase product in comparison to cells similarly transfected with control oligomers having the reverse sequence (Asp2-1 reverse & Asp2-2 reverse), see Figure 9. Correspondingly, cotransfection of Hu-Asp2 into mouse Neuro-2a cells with the APP-KK construct increased the formation of CTF99. (See Fig. 10.) This was further increased if Hu-Asp2 was coexpressed with APP-Sw-KK, a mutant form of APP containing the Swedish KM→NL mutation that increases β-secretase processing.

Effects of Asp2 on the production of Ab peptides from endogenously expressed APP isoforms were assessed in HEK293 cells transfected with a construct expressing Asp2 or with the empty vector after selection of transformants with the antibiotic G418. Aβ40 production was increased in cells transformed with the Asp2 construct in comparison to those transformed with the empty vector DNA. Aβ40 levels in conditioned medium collected from the Asp2 transformed and control cultures was 424 ± 45 pg /ml and 113 ± 58 pg/ml, respectively ($p<0.001$). Aβ42 release was below the limit of detection by the EIA, while the release of sAPPα was unaffected, 112 ± 8 ng/ml versus 111 ± 40 ng/ml. This further indicates that Asp2

acts directly or indirectly to facilitate the processing and release of Aβ from endogenously expressed APP.

Co-transfection of Hu-Asp2 with APP has little effect on Aβ40 production but increases Aβ42 production above background (Table 4). Addition of the di-lysine motif to the C-terminus of APP increases Aβ peptide processing about two fold, although Aβ40 and Aβ42 production remain quite low (352 pg/ml and 21 pg/ml, respectively). Cotransfection of Asp2 with APP-KK further increases both Aβ40 and Aβ42 production.

The APP V717-F mutation has been shown to increase γ-secretase processing at the Aβ42 cleavage site. Cotransfection of Hu-Asp2 with the APP-VF or APP-VF-KK constructs increased Aβ42 production (a two fold increase with APP-VF and a four-fold increase with APP-VF-KK, Table 4), but had mixed effects on Aβ40 production (a slight decrease with APP-VF, and a two fold increase with APP-VF-KK in comparison to the pcDNA cotransfection control. Thus, the effect of Asp2 on Aβ42 production was proportionately greater leading to an increase in the ratio of Aβ42/total Ab. Indeed, the ratio of Aβ42/total Aβ reaches a very high value of 42% in HEK293 cells cotransfected with Hu-Asp2 and APP-VF-KK.

Table 4

Results of cotransfecting Hu-Asp2 or pcDNA plasmid DNA with various APP constructs containing the V717-F mutation that modifies γ-secretase processing. Cotransfection with Asp2 consistently increases the ratio of Aβ42/total Aβ. Values tabulated are Aβ peptide pg/ml.

	pcDNA Cotransfection			Asp2 Cotransfection		
	Aβ40	Aβ42	Aβ42/Total	Aβ40	Aβ42	Aβ42/Total
APP	192±18	<4	<2%	188±40	8±10	3.9%
APP-VF	118±15	15±19	11.5%	85±7	24±12	22.4%
APP-KK	352±24	21±6	5.5%	1062±101	226±49	17.5%
APP-VF-KK	230±31	88±24	27.7%	491±35	355±36	42%

Example 9

Bacterial expression of human Asp2(a)

Expression of recombinant Hu-Asp2(a) in E. coli.

Hu-Asp2(a) can be expressed in *E. coli* after addition of N-terminal sequences such as a T7 tag (SEQ ID No. 21 and No. 22) or a T7 tag followed by a caspase 8 leader sequence (SEQ ID No. 23 and No. 24). Alternatively, reduction of the GC content of the 5' sequence by site directed mutagenesis can be used to increase the yield of Hu-Asp2 (SEQ ID No. 25 and No. 26). In addition, Asp2(a) can be engineered with a proteolytic cleavage site (SEQ ID No. 27 and No. 28). To produce a soluble protein after expression and refolding, deletion of the transmembrane domain and cytoplasmic tail, or deletion of the membrane proximal region, transmembrane domain, and cytoplasmic tail is preferred. Any materials (vectors, host cells, etc.) and methods described herein to express Hu-Asp2(a) should in principle be equally effective for expression of Hu-Asp2(b).

Methods

PCR with primers containing appropriate linker sequences was used to assemble fusions of Asp2(a) coding sequence with N-terminal sequence modifications including a T7 tag (SEQ ID Nos. 21 and 22) or a T7-caspase 8 leader (SEQ ID Nos. 23 and 24). These constructs were cloned into the expression vector pet23a(+) [Novagen] in which a T7 promoter directs expression of a T7 tag preceding a sequence of multiple cloning sites. To clone Hu-Asp2 sequences behind the T7 leader of pet23a+, the following oligonucleotides were used for amplification of the selected Hu-Asp2(a) sequence: #553=GTGGATCCACCCAGCACGGCATCCGGCTG (SEQ ID No. 35), #554=GAAAGCTTTCATGACTCATCTGTCTGTGGAATGTTG (SEQ ID No. 36) which placed BamHI and HindIII sites flanking the 5' and 3' ends of the insert, respectively. The Asp2(a) sequence was amplified from the full length Asp2(a) cDNA cloned into pcDNA3.1 using the Advantage-GC cDNA PCR [Clontech] following the manufacturer's supplied protocol using annealing & extension at 68°C in

a two-step PCR cycle for 25 cycles. The insert and vector were cut with BamHI and HindIII, purified by electrophoresis through an agarose gel, then ligated using the Rapid DNA Ligation kit [Boerhinger Mannheim]. The ligation reaction was used to transform the *E. coli* strain JM109 (Promega) and colonies were picked for the purification of plasmid (Qiagen,Qiaprep minispin) and DNA sequence analysis . For inducible expression using induction with isopropyl b-D-thiogalactopyranoside (IPTG), the expression vector was transferred into *E. coli* strain BL21 (Statagene). Bacterial cultures were grown in LB broth in the presence of ampicillin at 100 ug/ml, and induced in log phase growth at an OD600 of 0.6-1.0 with 1 mM IPTG for 4 hour at 37°C. The cell pellet was harvested by centrifugation.

To clone Hu-Asp2 sequences behind the T7 tag and caspase leader (SEQ ID Nos. 23 and 24), the construct created above containing the T7-Hu-Asp2 sequence (SEQ ID Nos. 21 and 22) was opened at the BamH1 site, and then the phosphorylated caspase 8 leader oligonucleotides #559=GATCGATGACTATCTCTGACTCTCCGCGTGAACAGGACG (SEQ ID No. 37), #560=GATCCGTCCTGTTCACGCGGAGAGTCAGAGATAGTCATC (SEQ ID No. 38) were annealed and ligated to the vector DNA. The 5' overhang for each set of oligonucleotides was designed such that it allowed ligation into the BamHI site but not subsequent digestion with BamHI. The ligation reaction was transformed into JM109 as above for analysis of protein expression after transfer to *E. coli* strain BL21.

In order to reduce the GC content of the 5' terminus of asp2(a), a pair of antiparallel oligos were designed to change degenerate codon bases in 15 amino acid positions from G/C to A/T (SEQ ID Nos. 25 and 26). The new nucleotide sequence at the 5' end of asp2 did not change the encoded amino acid and was chosen to optimize *E. Coli* expression. The sequence of the sense linker is 5' CGGCATCCGGCTGCCCCTGCGTAGCGGTCTGGGTGGTGCTCCACTGGGTCT GCGTCTGCCCCGGGAGACCGACGAA G 3' (SEQ ID No. 39). The sequence of the antisense linker is : 5' CTTCGTCGGTCTCCCGGGGCAGACGCAGACCCAGTGGAGCACCACCCAGA CCGCTACGCAGGGGCAGCCGGATGCCG 3' (SEQ ID No. 40). After annealing

the phosphorylated linkers together in 0.1 M NaCl-10 mM Tris, pH 7.4 they were ligated into unique Cla I and Sma I sites in Hu-Asp2 in the vector pTAC. For inducible expression using induction with isopropyl b-D-thiogalactopyranoside (IPTG), bacterial cultures were grown in LB broth in the presence of ampicillin at 100 ug/ml, and induced in log phase growth at an OD600 of 0.6-1.0 with 1 mM IPTG for 4 hour at 37°C. The cell pellet was harvested by centrifugation.

To create a vector in which the leader sequences can be removed by limited proteolysis with caspase 8 such that this liberates a Hu-Asp2 polypeptide beginning with the N-terminal sequence GSFV (SEQ ID Nos. 27 and 28), the following procedure was followed. Two phosphorylated oligonucleotides containing the caspase 8 cleavage site IETD, #571=5' GATCGATGACTATCTCTGACTCTCCGCTGGACTCTGGTATCGAAACCGACG (SEQ ID No. 41) and #572= GATCCGTCGGTTTCGATACCAGAGTCCAGCGGAGAGTCAGAGATAGTCATC (SEQ ID No. 42) were annealed and ligated into pET23a+ that had been opened with BamHI. After transformation into JM109, the purified vector DNA was recovered and orientation of the insert was confirmed by DNA sequence analysis.

The following oligonucleotides were used for amplification of the selected Hu-Asp2(a) sequence:
#573=5'AAGGATCCTTTGTGGAGATGGTGGACAACCTG, (SEQ ID No. 43)
#554=GAAAGCTTTCATGACTCATCTGTCTGTGGAATGTTG (SEQ ID No. 44)
which placed BamHI and HindIII sites flanking the 5' and 3' ends of the insert, respectively. The Hu-Asp2(a) sequence was amplified from the full length Hu-Asp2(a) cDNA cloned into pcDNA3.1 using the Advantage-GC cDNA PCR [Clontech] following the manufacturer's supplied protocol using annealing & extension at 68°C in a two-step PCR cycle for 25 cycles. The insert and vector were cut with BamHI and HindIII, purified by electrophoresis through an agarose gel, then ligated using the Rapid DNA Ligation kit [Boerhinger Mannheim]. The ligation reaction was used to transform the *E. coli* strain JM109 [Promega] and colonies were picked for the purification of plasmid (Qiagen,Qiaprep minispin) and DNA sequence

analysis . For inducible expression using induction with isopropyl b-D-thiogalactopyranoside (IPTG), the expression vector was transferred into *E. coli* strain BL21 (Statagene). Bacterial cultures were grown in LB broth in the presence of ampicillin at 100 ug/ml, and induced in log phase growth at an OD600 of 0.6-1.0 with 1 mM IPTG for 4 hour at 37°C. The cell pellet was harvested by centrifugation.

To assist purification, a 6-His tag can be introduced into any of the above constructs following the T7 leader by opening the construct at the BamHI site and then ligating in the annealed, phosphorylated oligonucleotides containing the six histidine sequence #565=GATCGCATCATCACCATCACCATG (SEQ ID No. 45), #566=GATCCATGGTGATGGTGATGATGC (SEQ ID No. 46). The 5' overhang for each set of oligonucleotides was designed such that it allowed ligation into the BamHI site but not subsequent digestion with BamHI.

Preparation of Bacterial Pellet:

36.34g of bacterial pellet representing 10.8L of growth was dispersed into a total volume of 200ml using a 20mm tissue homogenizer probe at 3000 to 5000 rpm in 2M KCl, 0.1M Tris, 0.05M EDTA, 1mM DTT. The conductivity adjusted to about 193mMhos with water. After the pellet was dispersed, an additional amount of the KCl solution was added, bringing the total volume to 500 ml. This suspension was homogenized further for about 3 minutes at 5000 rpm using the same probe. The mixture was then passed through a Rannie high-pressure homogenizer at 10,000psi.

In all cases, the pellet material was carried forward, while the soluble fraction was discarded. The resultant solution was centrifuged in a GSA rotor for 1 hour at 12,500 rpm. The pellet was resuspended in the same solution (without the DTT) using the same tissue homogenizer probe at 2,000 rpm. After homogenizing for 5 minutes at 3000 rpm, the volume was adjusted to 500ml with the same solution, and spun for 1 hour at 12,500 rpm. The pellet was then resuspended as before, but this time the final volume was adjusted to 1.5L with the same solution prior to homogenizing for 5 minutes. After centrifuging at the same speed for 30 minutes, this procedure was repeated. The pellet was then resuspended into about 150ml of cold water, pooling the pellets from the six centrifuge tubes used in the GSA rotor. The pellet has

homogenized for 5 minutes at 3,000 rpm, volume adjusted to 250ml with cold water, then spun for 30 minutes. Weight of the resultant pellet was 17.75g.

Summary: Lysis of bacterial pellet in KCl solution, followed by centrifugation in a GSA rotor was used to initially prepare the pellet. The same solution was then used an additional three times for resuspension/homogenization. . A final water wash/homogenization was then performed to remove excess KCl and EDTA.

Solublization of Recombinant Hu-Asp2(a):

A ratio of 9-10ml/gram of pellet was utilized for solubilizing the rHuAsp2L from the pellet previously described. 17.75g of pellet was thawed, and 150ml of 8M guanidine HCl, 5mM βME, 0.1% DEA, was added. 3M Tris was used to titrate the pH to 8.6. The pellet was initially resuspended into the guanidine solution using a 20 mm tissue homogenizer probe at 1000 rpm. The mixture was then stirred at 4°C for 1 hour prior to centrifugation at 12,500 rpm for 1 hour in GSA rotor. The resultant supernatant was then centrifuged for 30 minutes at 40,000 x g in an SS-34 rotor. The final supernatant was then stored at –20°C, except for 50 ml.

Immobilized Nickel Affinity Chromatography of Solubilized Recombinant Hu-Asp2(a):

The following solutions were utilized:

A) 6M Guanidine HCl, 0.1M NaP, pH 8.0, 0.01M Tris, 5mM βME, 0.5mM Imidazole

A') 6M Urea, 20mM NaP, pH 6.80, 50mM NaCl

B') 6M Urea, 20mM NaP, pH 6.20, 50mM NaCl, 12mM Imidazole

C') 6M Urea, 20mM NaP, pH 6.80, 50mM NaCl, 300mM Imidazole

Note: Buffers A' and C' were mixed at the appropriate ratios to give intermediate concentrations of Imidazole.

The 50ml of solubilized material was combined with 50ml of buffer A prior to adding to 100-125ml Qiagen Ni-NTA SuperFlow (pre-equilibrated with buffer A) in a 5 x

10cm Bio-Rad econo column. This was shaken gently overnight at 4°C in the cold room.

Chromatography Steps:

Drained the resultant flow through.
Washed with 50ml buffer A (collecting into flow through fraction)
Washed with 250ml buffer A (wash 1)
Washed with 250ml buffer A (wash 2)
Washed with 250ml buffer A'
Washed with 250ml buffer B'
Washed with 250ml buffer A'
Eluted with 250ml 75mM Imidazole
Eluted with 250ml 150mM Imidazole (150-1)
Eluted with 250ml 150mM Imidazole (150-2)
Eluted with 250ml 300mM Imidazole (300-1)
Eluted with 250ml 300mM Imidazole (300-2)
Eluted with 250ml 300mM Imidazole (300-3)

Chromatography Results:

The Hu-Asp(a) eluted at 75mM Imidazole through 300mM Imidazole. The 75mM fraction, as well as the first 150mM Imidazole (150-1) fraction contained contaminating proteins as visualized on Coomassie Blue stained gels. Therefore, fractions 150-2 and 300-1 will be utilized for refolding experiments since they contained the greatest amount of protein as visualized on a Coomassie Blue stained gel.

Refolding Experiments of Recombinant Hu-Asp2(a):

Experiment 1:

Forty ml of 150-2 was spiked with 1M DTT, 3M Tris, pH 7.4 and DEA to a final concentration of 6mM, 50mM, and 0.1% respectively. This was diluted suddenly

(while stirring) with 200ml of (4°C) cold 20mM NaP, pH 6.8, 150mM NaCl. This dilution gave a final Urea concentration of 1M. This solution remained clear, even if allowed to set open to the air at room temperature (RT) or at 4°C .
After setting open to the air for 4-5 hours at 4°C, this solution was then dialyzed overnight against 20 mM NaP, pH 7.4, 150 mM NaCl, 20% glycerol. This method effectively removes the urea in the solution without precipitation of the protein.

Experiment 2:

Some of the 150-2 eluate was concentrated 2x on an Amicon Centriprep, 10,000 MWCO, then treated as in Experiment 1. This material also stayed in solution, with no visible precipitation.

Experiment 3:

89ml of the 150-2 eluate was spiked with 1M DTT, 3M Tris, pH 7.4 and DEA to a final concentration of 6mM, 50mM, and 0.1% respectively. This was diluted suddenly (while stirring) with 445 ml of (4°C) cold 20 mM NaP, pH 6.8, 150 mM NaCl. This solution appeared clear, with no apparent precipitation. The solution was removed to RT and stirred for 10 minutes prior to adding MEA to a final concentration of 0.1 mM. This was stirred slowly at RT for 1 hour. Cystamine and $CuSO_4$ were then added to final concentrations of 1 mM and 10 μM respectively. The solution was stirred slowly at RT for 10 minutes prior to being moved to the 4°C cold room and shaken slowly overnight, open to the air.

The following day, the solution (still clear, with no apparent precipitation) was centrifuged at 100,000 x g for 1 hour. Supernatants from multiple runs were pooled, and the bulk of the stabilized protein was dialyzed against 20mM NaP, pH 7.4, 150 mM NaCl, 20% glycerol. After dialysis, the material was stored at -20°C.

Some (about 10 ml) of the protein solution (still in 1M Urea) was saved back for biochemical analyses, and frozen at -20°C for storage.

Example 10

Expression of Hu-Asp2 and Derivatives in Insect Cells

Any materials (vectors, host cells, etc.) and methods that are useful to express Hu-Asp2(a) should in principle be equally effective for expression of Hu-Asp2(b).

Expression by baculovirus infection.

The coding sequence of Hu-Asp2(a) and Hu-ASp2(b) and several derivatives were engineered for expression in insect cells using the PCR. For the full-length sequence, a 5'-sense oligonucleotide primer that modified the translation initiation site to fit the Kozak consensus sequence was paired with a 3'-antisense primer that contains the natural translation termination codon in the Hu-Asp2 sequence. PCR amplification of the pcDNA3.1(hygro)/Hu-Asp2(a) template was used to prepare two derivatives of Hu-Asp2(a) or Hu-Asp(b) that delete the C-terminal transmembrane domain (SEQ ID Nos. 29-30 and 50-51, respectively) or delete the transmembrane domain and introduce a hexa-histidine tag at the C-terminus (SEQ ID Nos. 31-32 and 52-53) respectively, were also engineered using PCR. The same 5'-sense oligonucleotide primer described above was paired with either a 3'-antisense primer that (1) introduced a translation termination codon after codon 453 (SEQ ID No. 3) or (2) incorporated a hexa-histidine tag followed by a translation termination codon in the PCR using pcDNA3.1(hygro)/Hu-Asp-2(a) as the template. In all cases, the PCR reactions were performed amplified for 15 cycles using *Pwo*I DNA polymerase (Boehringer-Mannheim) as outlined by the supplier. The reaction products were digested to completion with *BamH*I and *Not*I and ligated to *BamH*I and *Not*I digested baculovirus transfer vector pVL1393 (Invitrogen). A portion of the ligations was used to transform competent *E. coli* DH5_ cells followed by antibiotic selection on LB-Amp. Plasmid DNA was prepared by standard alkaline lysis and banding in CsCl to yield the baculovirus transfer vectors pVL1393/Asp2(a), pVL1393/Asp2(a)ΔTM and pVL1393/Asp2(a)ΔTM(His)$_6$. Creation of recombinant baculoviruses and infection of sf9 insect cells was performed using standard methods.

Expression by transfection

Transient and stable expression of Hu-Asp2(a)ΔTM and Hu-Asp2(a)ΔTM(His)$_6$ in High 5 insect cells was performed using the insect expression vector pIZ/V5-His. The DNA inserts from the expression plasmids vectors pVL1393/Asp2(a), pVL1393/Asp2(a)ΔTM and pVL1393/Asp2(a)ΔTM(His)$_6$ were excised by double digestion with *BamHI* and *NotI* and subcloned into *BamHI* and *NotI* digested pIZ/V5-His using standard methods. The resulting expression plasmids, referred to as pIZ/Hu-Asp2ΔTM and pIZ/Hu-Asp2ΔTM(His)$_6$, were prepared as described above.

For transfection, High 5 insect cells were cultured in High Five serum free medium supplemented with 10 µg/ml gentamycin at 27°C in sealed flasks. Transfections were performed using High five cells, High five serum free media supplemented with 10 µg/ml gentamycin, and InsectinPlus liposomes (Invitrogen, Carlsbad, CA) using standard methods.

For large scale transient transfections, 1.2 x 10^7 high five cells were plated in a 150 mm tissue culture dish and allowed to attach at room temperature for 15-30 minutes. During the attachment time the DNA/ liposome mixture was prepared by mixing 6 ml of serum free media, 60 µg Hu-Asp2(a)ΔTM/pIZ (+/- His) DNA and 120 µl of Insectin Plus and incubating at room temperature for 15 minutes. The plating media was removed from the dish of cells and replaced with the DNA/liposome mixture for 4 hours at room temperature with constant rocking at 2 rpm. An additional 6 ml of media was added to the dish prior to incubation for 4 days at 27 °C in a humid incubator. Four days post transfection the media was harvested, clarified by centrifugation at 500 x g, assayed for Hu-Asp2(a) expression by Western blotting. For stable expression, the cells were treated with 50 µg/ml Zeocin and the surviving pool used to prepared clonal cells by limiting dilution followed by analysis of the expression level as noted above.

Purification of Hu-Asp2(a)ΔTM and Hu-Asp2(a)ΔTM(His)$_6$

Removal of the transmembrane segment from Hu-Asp2(a) resulted in the secretion of the polypeptide into the culture medium. Following protein production

by either baculovirus infection or transfection, the conditioned medium was harvested, clarified by centrifugation, and dialyzed against Tris-HCl (pH 8.0). This material was then purified by successive chromatography by anion exchange (Tris-HCl, pH 8.0) followed by cation exchange chromatography (Acetate buffer at pH 4.5) using NaCl gradients. The elution profile was monitored by (1) Western blot analysis and (2) by activity assay using the peptide substrate described in Example 12. For the Hu-Asp2(a)ΔTM$(His)_6$, the conditioned medium was dialyzed against Tris buffer (pH 8.0) and purified by sequential chromatography on IMAC resin followed by anion exchange chromatography.

Amino-terminal sequence analysis of the purified Hu-Asp2(a)ΔTM$(His)_6$ protein revealed that the signal peptide had been cleaved [TQHGIRLPLR, corresponding to SEQ ID NO: 32, residues 22-3].

Example 11

Expression of Hu-Asp2(a) and Hu-Asp(b) in CHO cells

The materials (vectors, host cells, etc.) and methods described herein for expression of Hu-Asp2(a) are intended to be equally applicable for expression of Hu-Asp2(b).

Heterologous expression of Hu-Asp-2(a) in CHO-K1 cells

The entire coding sequence of Hu-Asp2(a) was cloned into the mammalian expression vector pcDNA3.1(+)Hygro (Invitrogen, Carlsbad, CA) which contains the CMV immediate early promoter and bGH polyadenylation signal to drive over expression. The expression plasmid, pcDNA3.1(+)Hygro/Hu-Asp2(a), was prepared by alkaline lysis and banding in CsCl and completely sequenced on both strands to verify the integrity of the coding sequence.

Wild-type Chinese hamster ovary cells (CHO-K1) were obtained from the ATCC. The cells were maintained in monolayer cultures in α-MEM containing 10% FCS at 37°C in 5% CO_2. Two 100 mm dishes of CHO-K1 cells (60% confluent) were transfected with pcDNA3.1(+)/Hygro alone (mock) or pcDNA3.1(+)Hygro/Hu-Asp2(a) or pcDNA3.1(+)Hygro/Hu-Asp2(b) using the

cationic liposome DOTAP as recommended by the supplier (Roche, Indianapolis, IN). The cells were treated with the plasmid DNA/liposome mixtures for 15 hours and then the medium replaced with growth medium containing 500 Units/ml hygromycin B. In the case of pcDNA3.1(+)Hygro/Hu-Asp2(a) or (b) transfected CHO-K1cells, individual hygromycin B-resistant cells were cloned by limiting dilution. Following clonal expansion of the individual cell lines, expression of Hu-Asp2(a) or Hu-Asp2(b) protein was assessed by Western blot analysis using a polyclonal rabbit antiserum raised against recombinant Hu-Asp2 prepared by expression in *E. coli*. Near confluent dishes of each cell line were harvested by scraping into PBS and the cells recovered by centrifugation. The cell pellets were resuspended in cold lysis buffer (25 mM Tris-HCl (pH 8.0)/5 mM EDTA) containing protease inhibitors and the cells lysed by sonication. The soluble and membrane fractions were separated by centrifugation (105,000 x g, 60 min) and normalized amounts of protein from each fraction were then separated by SDS-PAGE. Following electrotransfer of the separated polypeptides to PVDF membranes, Hu-Asp-2(a) or Hu-Asp2(b) protein was detected using rabbit anti-Hu-Asp2 antiserum (1/1000 dilution) and the antibody-antigen complexes were visualized using alkaline phosphatase conjugated goat anti-rabbit antibodies (1/2500). A specific immunoreactive protein with an apparent Mr value of 65 kDa was detected in pcDNA3.1(+)Hygro/Hu-Asp2 transfected cells and not mock-transfected cells. Also, the Hu-Asp2 polypeptide was only detected in the membrane fraction, consistent with the presence of a signal peptide and single transmembrane domain in the predicted sequence. Based on this analysis, clone #5 had the highest expression level of Hu-Asp2(a) protein and this production cell lines was scaled up to provide material for purification.

Purification of recombinant Hu-Asp-2(a) from CHO-K1/Hu-Asp2 clone #5

In a typical purification, clone #5 cell pellets derived from 20 150 mm dishes of confluent cells, were used as the starting material. The cell pellets were resuspended in 50 ml cold lysis buffer as described above. The cells were lysed by polytron homogenization (2 x 20 sec) and the lysate centrifuged at 338,000 x g for 20 minutes. The membrane pellet was then resuspended in 20 ml of cold lysis buffer

containing 50 mM β-octylglucoside followed by rocking at 4 °C for 1 hour. The detergent extract was clarified by centrifugation at 338,000 x g for 20 minutes and the supernatant taken for further analysis.

The β-octylglucoside extract was applied to a Mono Q anion exchange column that was previously equilibrated with 25 mM Tris-HCl (pH 8.0)/50 mM β-octylglucoside. Following sample application, the column was eluted with a linear gradient of increasing NaCl concentration (0-1.0 M over 30 minutes) and individual fractions assayed by Western blot analysis and for β-secretase activity (see below). Fractions containing both Hu-Asp-2(a) immunoreactivity and β-secretase activity were pooled and dialyzed against 25 mM NaOAc (pH 4.5)/50 mM β-octylglucoside. Following dialysis, precipitated material was removed by centrifugation and the soluble material chromatographed on a MonoS cation exchange column that was previously equilibrated in 25 mM NaOAc (pH 4.5)/ 50 mM β-octylglucoside. The column was eluted using a linear gradient of increasing NaCl concentration (0-1.0 M over 30 minutes) and individual fractions assayed by Western blot analysis and for β-secretase activity. Fractions containing both Hu-Asp2 immunoreactivity and β-secretase activity were combined and determined to be >95% pure by SDS-PAGE/Coomassie Blue staining.

The same methods were used to express and purify Hu-Asp2(b).

Example 12

Assay of Hu-Asp2 β-secretase activity using peptide substrates

β-secretase assay

Recombinant human Asp2(a) prepared in CHO cells and purified as described in Example 11 was used to assay Asp2(a) proteolytic activity directly. Activity assays for Asp2(a) were performed using synthetic peptide substrates containing either the wild-type APP β-secretase site (SEVKM↓DAEFR), the Swedish KM→NL mutation (SEVNL↓DAEFR), or the Aβ40 and 42 γ-secretase sites (RRGGVV↓IA↓TVIVGER). Reactions were performed in 50 mM 2-[N-morpholino]ethane-sulfonate ("Na-MES," pH 5.5) containing 1% β-octylglucoside, 70 mM peptide substrate, and recombinant

Asp2(a) (1-5 μg protein) for various times at 37°C. The reaction products were quantified by RP-HPLC using a linear gradient from 0-70 B over 30 minutes (A=0.1% TFA in water, B=0.1%TFA/10%water/90%AcCN). The elution profile was monitored by absorbance at 214 nm. In preliminary experiments, the two product peaks which eluted before the intact peptide substrate, were confirmed to have the sequence DAEFR and SEVNL using both Edman sequencing and MADLI-TOF mass spectrometry. Percent hydrolysis of the peptide substrate was calculated by comparing the integrated peak areas for the two product peptides and the starting material derived from the absorbance at 214 nm. The sequence of cleavage/hydrolysis products was confirmed using Edman sequencing and MADLI-TOF mass spectrometry.

The behavior of purified Asp2(a) in the proteolysis assays was consistent with the prior anti-sense studies which indicated that Asp2(a) possesses β-secretase activity. Maximal proteolysis was seen with the Swedish β-secretase peptide, which, after 6 hours, was about 10-fold higher than wild type APP.

The specificity of the protease cleavage reaction was determined by performing the β-secretase assay in the presence of 8 μM pepstatin A and the presence of a cocktail of protease inhibitors (10 μM leupeptin, 10 μM E64, and 5 mM EDTA). Proteolytic activity was insensitive to both the pepstatin and the cocktail, which areinhibitors of cathepsin D (and other aspartyl proteases), serine proteases, cysteinyl proteases, and metalloproteases, respectively.

Hu-Asp2(b) when similarly expressed in CHO cells and purified using identical conditions for extraction with β-octylglucoside and sequential chromatography over Mono Q and Mono S also cleaves the Swedish β-secretase peptide in proteolysis assays using identical assay conditions.

Collectively, this data establishes that both forms of Asp2 (Hu-Asp2(a) and Hu-Asp2(b)) act directly in cell-free assays to cleave synthetic APP peptides at the β-secretase site, and that the rate of cleavage is greatly increased by the Swedish KM→NL mutation that is associated with Alzheimer's disease.

An alternative β-secretase assay utilizes internally quenched fluorescent substrates to monitor enzyme activity using fluorescence spectroscopy in a single sample or multiwell format. Each reaction contained 50 mM Na-MES (pH 5.5), peptide substrate MCA-EVKMDAEF[K-DNP] (BioSource International) (50 μM) and purified Hu-Asp-2 enzyme. These components were equilibrated to 37 °C for various times and the reaction initiated by addition of substrate. Excitation was performed at 330 nm and the reaction kinetics were monitored by measuring the fluorescence emission at 390 nm. To detect compounds that modulate Hu-Asp-2 activity, the test compounds were added during the preincubation phase of the reaction and the kinetics of the reaction monitored as described above. Activators are scored as compounds that increase the rate of appearance of fluorescence while inhibitors decrease the rate of appearance of fluorescence.

Example 13

Demonstration that Asp1 processes APP at the α-secretase site

Increased expression of an α-secretase candidate gene in human cells would be expected to increase basal release of sAPPα and to decrease release of Aβ peptides. This the effect was observed when full length human Asp1 is co-expressed with APP in HEK293 cells. The experiment utilized the APP 695 amino acid isoform which had been modified by the addition of a pair of lysine residues to the C-terminus (APP-KK). The C-terminal di-lysine motif increases the intracellular half-life of glycosylated APP and consequently the production of both sAPPα and Aβ. As shown in Table 5, cotransfection of HEK293 cells with APP-KK with Asp1 increased the production of sAPPα by 3.5 fold ($p<0.001$) and decreased the production of Aβ40 by 2.8 fold. Thus, Asp1 acts directly or indirectly to facilitate constitutive α-secretase cleavage and this effect is competitive with the amyloidogenic processing of APP to Aβ peptides. This implies that mutations or genetic polymorphisms in Asp1 may affect Aβ production by affecting the balance between the competing pathways for constitutive α-secretase cleavage and Aβ peptide production.

Table 5.

Asp1 stimulates basal release of sAPPα from HEK293 cells after cotransfection with APP-KK.

Transfection	sAPPα μg/ml	Fold Increase	Aβ40 pg/ml	Fold Decrease
Asp1	3.5 + 1.1	+3.5	113 + 7	-2.8
pcDNA	1.0 + 0.2		321 + 18	

Specific methods used were as follows. The full length Asp1 cDNA was cloned into the vector pcDNA3.1/hygro+(Invitrogen) for transfection studies as previously described (Yan *et al.*, (1999) *Nature 402:* 533-537). The APP-KK cDNA was cloned into the vector pIRES (Clontech) also as previously described. HEK293 cells were transfected with expression constructs using the Lipofectamine Plus reagent from Gibco/BRL. Cells were seeded in 24 well tissue culture plates to a density of 70-80% confluence. Four wells per plate were transfected with 2 μg DNA (3:1, APP:Asp1 or empty pcDNA3.1./hygro+ vector), 8μl Plus reagent, and 4μl Lipofectamine in OptiMEM. OptiMEM was added to a total volume of 1 ml, distributed 200 μl per well and incubated 3 hours. Care was taken to hold constant the ratios of the two plasmids used for cotransfection as well as the total amount of DNA used in the transfection. The transfection media was replaced with DMEM supplemented with 10% FBS and NaPyruvate, with antibiotic/antimycotic and the cells were incubated under normal conditions (37°, 5% CO_2) for 48 hours. The conditioned media were removed to polypropylene tubes and stored at –80°C until assayed for the content of sAPPα or Aβ40/Aβ42 by enzyme-linked immunosorbent assay (EIA) as described above in Example 6. The Aβ EIA followed the protocol of Pirttila *et al.* (*Neuro. Lett.* (1999) 249*:* 21-4) using the 6E10 monoclonal antibody (Senetek) as a capture antibody and biotinylated rabbit antiserum 162 or 165 (New York State Institute for Basic Research, Staten Island, NY) for detection of Aβ40 and Aβ42, respectively. The 6E10 antibody recognizes residues 1-16 of the Aβ peptide. The sAPPα EIA used LN27 antibody as a capture antibody and biotinylated 6E10 for

detection as described previously (Yan *et al.*, (1999) *supra.*). The LN27 antibody recognized the first 20 amino acids of the human APP peptide.

Increased α-secretase activity and concomitant reduction of Aβ production *in vivo* represents an effect that may be desirable for the prevention, treatment (*e.g.*, to show the progression of), or cure of Azheimer's disease. Thus, the activities demonstrated in this example provide an indication that modulators of Asp1 activity, that achieve the same effects *in vivo*, will have utility for Alzheimer's disease therapy. Screening methods for such modulators are contemplated as an aspect of the invention.

Example 14

Expression of Pre-pro-Hu-Asp1 and Derivatives in Insect Cells

Expression of hu-Asp-1TM(His)^6 by baculovirus infection.

The coding sequence of pre-pro-Hu-Asp1 was engineered for production as a soluble, secreted form by insect cells. PCR primers were designed to (1) delete the predicted transmembrane domain and cytoplasmic tail of Asp1 and (2) to introduce a Kozak consensus sequence for efficient translational initiation. The primers sequences were are follows: sense CGCTTTAAGCTTGCCACCATGGGCGCA CTGGCCCGGGCG (SEQ ID NO: 74) and antisense CGCTTTCTCGAGCTAA TGGTGATGGTGATGGTGCCACAAAATGGGCTCGCTCAAAGA (SEQ ID NO: 75) which replaced the deleted C-terminal transmembrane and cytoplasmic domains with a hexahistidine purification tag.

PCR reactions were carried out with 100 ng of full length Asp1 pcDNA 3.1 hygro+ construct, 200 M NTPs, 300 nM of each primer, 1x reaction buffer containing 2 mM $MgSO_4$, and 5 units of Pwo I DNA polymerase (Roche Biochemicals). The reactions were cycled under the following conditions: 94°C for 5 minutes followed by 15 cycles of 94°C for 30 seconds and 72°C for 30 seconds, and then a final extension reaction at 72°C for 10 minutes. The predicted amino acid sequence of this PCR generated derivative (denoted as Asp-1ΔTM(His)$_6$) is set out as SEQ ID NO: 66.

The reaction product was digested to completion with HindIII-XhoI and ligated into the expression vector pIB (Invitrogen) to yield the pIB/Asp-1ΔTM(His)$_6$ construct. Creation of recombinant baculovirus and infection of sf9 insect cells was performed using standard methods known in the art. Sf9 cells were transfected with either the pIB vector alone or the pIB/Asp-1ΔTM(His)$_6$ construct utilizing Insectin Plus reagent (Invitrogen) according to the manufacturer's instructions. After the transfection, the cells were cultured in High Five serum-free media (Invitrogen) for 3 days. Subsequently, the conditioned medium was harvested and subjected to Western blot analysis. This analysis revealed specific expression and secretion of immunoreactive Asp-1ΔTM(His)$_6$ polypeptide into the extracellular medium. The secreted proteins were detected on the Western blot with either the India probe (Pierce Chemicals) specific for the hexahistidine sequence tag or using a rabbit polyclonal antiserum. The polyclonal antisera (denoted as UP-199) was generated by injecting rabbits with recombinant Asp-1ΔTM(His)$_6$ (SEQ ID NO: 66). This recombinant peptide was prepared by heterologous expression in *E.coli*. The UP-199 antibody recognizes the processed form of Asp-1ΔTM.

Direct analysis with the polyclonal antiserum (UP-199) revealed an immunoreactive band of the expected molecular weight (50 kDa) only in pIB/Asp-1ΔTM(His)$_6$ transfected cells. This signal was significantly enhanced in concentrated conditioned medium. A similar pattern was obtained using the India probe. No signal was detected in conditioned medium derived from mock-transfected cells using either UP-199 antisera or the India probe.

Based on this result, transient and stable transfections of the pIB/Asp-1ΔTM(His)$_6$ construct in sf9 insect cells were carried out as described above. Four days post transient transfection, the culture medium was collected to provide material for further characterization. In parallel, sf9 cells were stably transfected with the pIB/Asp-1ΔTM(His)$_6$ construct and cultured in High Five serum-free medium (Invitrogen) supplemented with 50 μg/ml blasticidin for approximately 2 weeks. After blasticidin selection, the resistant pool of cells was expanded to provide a stable source of conditioned medium for Asp-1ΔTM(His)$_6$ purification.

Purification of recombinant Asp-1ΔTM(His)$_6$

Conditioned media, from either transient or stably transfected sf9 cells, were concentrated approximately 10-fold using a stirred cell concentrator equipped with a 30,000 MWCO membrane (Spectrum Medical Industries). This concentrate was then subjected to ammonium sulfate precipitation to further concentrate the sample and provide partial purification. Material precipitating between 0-40% saturation was discarded and the resulting supernatant was brought to 80% saturation. Western blot analysis of the various ammonium sulfate precipitated fractions revealed that the majority of the immunoreactive material was contained within the 40-80% ammonium sulfate pellet. As a result, this material was subjected to further purification.

The 40-80% ammonium sulfate pellet was redisolved in approximately 1/20 the original volume of Ni+-NTA loading buffer (25 mM Tris-HCl (pH 8.5)/0.5 M NaCl/10 mM imidazole). Subsequently, the sample was applied to a Ni+-NTA column previously equilibrated in Ni+-NTA buffer. Following sample application, the column was washed with starting buffer (25 mM Tris-HCl (ph 8.5)/ 0.5 M NaCl/ 20 mM imidazole) until the A^{280nm} of the column effluent returned to zero. After washing, the bound recombinant protein was eluted off the column with a linear gradient of Ni+-NTA buffer containing increasing concentrations (10 mM, 50 mM, 100 mM, 250 mM, and 500 mM) of imidazole. The elution profile was monitored by Western blot analysis using the UP-199 antiserum. Immunoreactive Asp-1ΔTM(His)$_6$ was detected in the column load and eluted at 50 mM imidazole. NuPAGE gel analysis of the 50 mM imidazole fraction demonstrated a purity of Asp-1ΔTM(His)$_6$ of approximately 50%, therefore further purification was required.

The positive fractions, eluted off the Ni+-NTA column, were then pooled (denoted as post-IMAC pool), concentrated using a YM30 membrane (Amicron), and dialyzed with 25 mM Tris-HCl (pH 8.0). The dialyzed post-IMAC pool was fractionated by MonoQ anion exchange chromatography (Amersham-Pharmacia

Biotech) gradient elution containing increasing concentrations (0 -0.5 M) of NaCl (Buffer A: 25 mM Tris-HCl (pH 8.0) and Buffer B: 25 mM Tris-HCl (pH 8.0)/ 0.5 M NaCl). The elution profile was determined by Western blot analysis which indicated immunoreactive fractions as those displaying immunoreactivity with the UP-199 antisera. NuPAGE gel analysis with silver staining demonstrated that the material prepared in this manner was >90% pure. The immunoactive fractions eluted off the MonoQ anion exchange column were pooled, dialyzed with 25 mM HEPES-Na+ (pH 8.0), and stored at 4°C until further analysis.

Acid-activation of recombinant Asp-1 TM(His)$_6$

Recombinant Asp-1ΔTM(His)$_6$ migrated with an apparent molecular weight of 50 kD. Direct N-terminal sequence analysis carried out by automated Edman degradation for 20 cycles revealed a unique sequence beginning at Glu^3 (SEQ ID NO: 67), confirming the identity of the recombinant protein. Computer assisted prediction of the signal peptidase cleavage site indicated that the pro-form should initiate at Ala^1, suggesting either an unusual processing site by the signal peptidase during secretion or an additional processing step that removes an additional two amino acid residues.

To investigate the mechanism of pro-Asp-1ΔTM(His)$_6$ activation, aliquots of the purified protein were incubated in various acidic environments with pH values ranging from 3.0-8.0 at 37°C for 2 hours. Subsequently, the recombinant proteins were analyzed by Western blot. A faster migrating polypeptide species was detected after incubation at pH values of 4.0, 4.5 and 5.0. The polypeptide migration was unaltered after incubation in environments which were either more acidic (pH 3.0 and 3.5) or more basic (pH 6.0, 7.0, and 8.0). Sequence analysis of this faster migrating species revealed that it initiated exclusively at Ala^{43}, consistent with removal of a 42 amino acid residue segment of the pro-peptide that was induced by treatment of the pro-enzyme at pH 4.5. The predicted amino acid sequence of the acid processed form of Asp-1ΔTM(His)$_6$ is set out as SEQ ID NO: 68.

To purify the acid-activated form of Asp-1ΔTM(His)$_6$, the Asp-1ΔTM(His)$_6$ post-IMAC pool (generated as described above) was dialyzed to pH 4.5 and then

subjected to affinity chromatography on either pepstatin A agarose or sulfolink-PHA-292593E. Following sample application, the column was washed with 25 mM NaOAc (pH 4.5) and eluted with 50 mM Na-BO_3 (pH 9.5). The positive fractions eluted off the columns were dialyzed with 25 mM Hepes-Na (pH 7.5) overnight at 4°C which resulted in quantitative conversion of the pro-enzyme to the acid-processed form (SEQ ID NO: 68) described above. Western blot analysis of the elution profile revealed quantitative retention of immunoreactive Asp-1ΔTM$(His)_6$ on both affinity resins as evidenced by the lack of Asp-1ΔTM$(His)_6$ in the unbound fraction as detected by UP-199 immunoreactivity on a Western blot. Step elution 50 mM $NaBO_3$ at pH 9.5 resulted in elution of immunoreactive Asp-1 TM$(His)_6$, with variable recovery.

Comparison of the properties of the recombinant soluble catalytic domain of Asp1 with the properties determined for Asp2 (see Example 10) revealed a number of significant differences. Processing of the pre-pro forms of either enzyme is distinct, with Asp1 undergoing efficient processing by the signal peptidase and additional processing to remove two additional amino acid residues from the N-terminus. Further processing of the pro-form of Asp1 was not detected in neutral pH. In contrast; recombinant Asp2 produced, under similar conditions, yields an eqimolar mixture of the pro-form and a processed form that has 24 amino acid residues of the pro-segment removed.

Another distinction between the processing of these two enzymes involves processing initiated by acid-treatment. Systematic analysis of acid-induced processing of pro-Asp2 revealed that the purified polypeptide did not self-process. In contrast, acid dependent processing of pro-Asp1 was readily demonstrated (as described above). Alignment of the self-processing site in Asp1 with the processing site in Asp2 revealed that these two enzymes are processed at the same position, which is a different method of processing as compared with that of other known human aspartyl proteases.

In addition to providing valuable information about Asp1 activity, the discovery of a site of apparent autocatalytic processing of Asp1 provides an indication

of a peptide sequences (surrounding Ala[43]) that could be useful for performing screening assays to identify modulators of Asp1 activity. This idea is explored in greater detail in Example 15.

Example 15

Development of an enzymatic assay for Asp-1ΔTM(His)$_6$

The relationship between Asp1 and APP processing was explored by determining if APP α-secretase, APP β-secretase, or APP γ-secretase peptide substrates were cleaved by recombinant Asp-1ΔTM(His)$_6$. These peptide substrates included the α-secretase specific substrates Aβ_{10-20} and Aβ_{12-28}, the β-secretase specific substrates PHA-95812E (SEVKMDAEFR; SEQ ID NO: 64) and PHA-247574E (SEVNLDAEFR; SEQ ID NO: 63), and γ-secretase specific substrate PHA-179111E (RRGGVVIATVIVGER; SEQ ID NO: 76). Each reaction consisted of incubating a peptide substrate (100 nM) with recombinant Asp-1ΔTM(His)$_6$ for 15 hours at pH 4.5 at 37°C. Reaction products were quantified by RP-HPLC at $A^{214\ nm}$. The elution profiles for Asp-1ΔTM(His)$_6$ were compared to those obtained from parallel Asp1 experiments. The identity of the cleavage products was determined by MADLI-TOF mass spectrometry. Table 6 summarizes the Asp1 substrates and indicates the cleavage site.

Table 6

Substrate Preferences of Asp-1ΔTM

P4	P3	P2	P1		P1′	P2′	P3′	P4′		SEQ ID NO:
G	L	A	L	↓	A	L	E	P	Self Activation	69
E	V	K	M	↓	D	A	E	F	β-Secretase, WT	70
E	V	N	L	↓	D	A	E	F	β-Secretase, Sw	71
L	V	F	F	↓	A	E	D	V	Aβ_{12-28} (α-Secretase)	72
K	L	V	F	↓	F	A	E	D	Aβ_{12-28} (α-Secretase)	73

The peptides in Table 6 are described using the nomenclature by Schechter and Berger (*Biochem. Biophys. Res. Commun.* 27:157(1967) and *Biochem. Biophys. Res. Commun.* 32:898 (1968)), in which the amino acid residues in the peptide substrate that undergo the cleavage are defined as $P_1 \ldots P_n$ toward the N-terminus and $P_1' \ldots P_n'$ toward the C-terminus. Therefore, the scissile bond is between the P_1 and the P_1' residue of the peptide subunits and is denoted herein throughout with a hyphen between the P_1 and the P_1'.

Digestion of the α-secreatse substrate (A_{12-28}) revealed two Asp1 cleavage sites. The major product was cleaved at Phe^{20}ıAla^{21} and the minor product was cleaved at Phe^{19}ıPhe^{20} (referring to the numbering convention in the APP Aβ) peptide. Analysis of the cleavage products obtained from the β-secretase peptide substrates revealed that both the wild-type (PHA-95812E) and the Swedish mutation (PHA-247574E) substrates were hydrolyzed exclusively at the β-secretase site. Also, the relative rates of Asp-1-dependent hydrolysis of the β-secretase peptide substrate containing the Swedish mutation was cleaved at least 10-times faster than the corresponding wild-type peptide. Conversion of the γ-secretase peptide substrate was not detected under these reaction conditions.

Measurement of the cleavage of the α-secretase and β-secretase substrates can also be carried out with substrates comprising detectable labels such as radioactive, enzymati , chemiluminescent or flourescent labels. For example, the peptide substrates could comprise internally quenched labels that result in increased detectability after cleavage of the peptide substrates due to separation of the labels upon cleavage. The peptide substrates can be modified to have attached a paired fluorprobe and quencher such as 7-amino-4-methyl courarin and dinitrophenol, respectively.

This example illustrates the α-secretase and β-secretase activity exhibited by Asp-1, confirming the APP processing activity of Asp1 indicating, *e.g.*, in Examples 7 and 13. The substrates described herein may be used in combination with recombinant Asp1 to measure Asp1 proteolytic activity at the α-secretase and β-secretase

processing sites. These substrates are useful in screening assays for identification of modulators of Asp1 proteolytic activity.

In particular, production of Aβ species through the processing of APP at β-and γ-secretase sites may play a central role in Alzheimer's disease pathogenesis, and processing at the α-secretase site may have a protective role and may prevent Aβ production. Thus, a therapeutic and/or prophylactic indication exists for molecules that can increase Asp1 α-secretase activity and/or decrease Asp1 β-secretase activity *in vivo*. The present invention includes screening assays for such modulators, and the foregoing substrate peptides are useful in such assays.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention. The entire disclosure of all publications cited herein are hereby incorporated by reference.

What is claimed is:

1. A method for assaying hu-Asp1 α-secretase activity comprising the steps of:

(a) contacting hu-Asp1 protein with an amyloid precursor protein (APP) substrate, wherein said substrate contains an α-secretase cleavage site; and

(b) measuring cleavage of the APP substrate at the α-cleavage site, thereby assaying hu-Asp1 α-secretase activity.

2. A method according to claim 1, wherein the hu-Asp1 protein comprises a polypeptide produced in cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes hu-Asp1 or a fragment thereof that retains Asp1 α-secretase activity.

3. A method of claim 2, wherein the hu-Asp1 protein is purified and isolated from said cell.

4. A method according to claims 2 or 3, wherein the nucleotide sequence encodes a polypeptide that comprises the hu-Asp1 amino acid sequence set forth in SEQ ID NO: 2 or a fragment thereof, wherein said fragment retains α-secretase activity.

5. A method according to any one of claims 2-4, wherein the polynucleotide sequence encodes a hu-Asp1 amino acid sequence lacking the transmembrane amino acids 469-492 of SEQ ID NO: 2.

6 A method according to claim 5, wherein the polynucleotide sequence encodes a hu-Asp1 amino acid sequence further lacking the cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2.

7. A method according to any one of claims 1-6, wherein the hu-Asp1 amino acid sequence lacks amino terminal amino acids 1-62 of SEQ ID NO: 2.

8. A method according to claims 1 or 2, wherein the contacting step comprises growing a cell transfected or transformed with a polynucleotide encoding hu-Asp1 protein or a fragment thereof that retains hu-Asp1 α-secretase activity, wherein the cell is grown under conditions in which the cell expresses the hu-Asp1 protein in the presence of the APP substrate.

9. A method of claim 8, wherein said cell expresses a polynucleotide encoding an APP substrate containing an α-secretase cleavage site, and wherein the contacting step comprises growing the cell under conditions in which the cell expresses the hu-Asp1 protein and the APP substrate.

10. A method of any one of claims 1-9, wherein the APP substrate α-secretase cleavage site comprises the amino acid sequence LVFFAEDF or KLVFFAED.

11. A method of any one of claims 1-10, wherein the APP substrate comprises a human APP isoform and further comprises a carboxy-terminal di-lysine.

12. A method of claims 10 or 11, wherein the APP substrate comprises a detectable label.

13. A method of claim 12, wherein the detectable label is selected from the group consisting of radioactive labels, chemiluminescent labels, enzymatic labels, chemiluminescent labels and flourescent labels.

14. A method of any one of claims 1-13, wherein the APP substrate comprises a human APP isoform and the determining step comprises measuring the production of amyloid alpha peptide (sAPPα).

15. A method of any one of claims 1-14, wherein the method further comprises the steps of:

(a) determining the level of hu-Asp1 α-secretase activity in the presence and absence of a modulator of hu-Asp1 α-secretase activity; and

(b) comparing the hu-Asp1 α-secretase activity in the presence and absence of the modulator, wherein modulators that increase hu-Asp1 α-secretase activity are identified as candidate Alzheimer's disease therapeutics.

16. A method of claim 15, wherein the method further comprises a step of treating Alzheimer's disease with said candidate Alzheimer's disease therapeutic.

17. A composition comprising a candidate Alzheimer's disease therapeutic identified by the method of claim 15.

18. A hu-Aspl protease substrate peptide or fragment thereof, wherein said peptide comprises an amino acid sequence consisting of fifty or fewer amino acids, said amino acid sequence including the hu-Aspl cleavage site having the amino acid sequence GLALALEP

19. The substrate of claim 18, wherein the substrate comprises a detectable label.

20. The substrate of claim 19, wherein the detectable label is selected from the group consisting of radioactive labels, enzymatic labels, chemiluminescent labels and flourescent labels.

21. A method for assaying hu-Asp1 proteolytic activity comprising the steps of:

(a) contacting hu-Asp1 protein with an hu-Aspl substrate according to claim 18, 19, or 20 under acidic conditions, and

(b) determining the level of hu-Asp1 proteolytic activity.

22. A method according to claim 20, wherein the hu-Asp1 protein comprises a polypeptide produced in cell transformed or transfected with a polynucleotide comprising the nucleotide sequence that encodes hu-Asp1.

23. A method of claim 21, wherein the hu-Aspl protein is purified and isolated from said cell.

24. A method according to claims 22 and 23, wherein the nucleotide sequence encodes a polypeptide that comprises the hu-Asp1 amino acid sequence set forth in SEQ ID NO: 2 or a fragment thereof, wherein said fragment retains proteolytic activity.

25. A purified polynucleotide comprising a nucleotide sequence encoding a polypeptide that comprises a fragment of a hu-Asp-1 protein, wherein said nucleotide sequence lacks the sequence that encodes the transmembrane domain of said hu-Asp1 protein, and wherein the hu-Asp1 polypeptide fragment encoded by said polynucleotide has hu-Asp1 α-secretase activity.

26. A polynucleotide of claim 25, wherein the polypeptide comprises a fragment of the hu-Asp1 amino acid sequence set forth in SEQ ID NO: 2, and wherein the polypeptide lacks the transmembrane domain amino acids 469-492 of SEQ ID NO: 2.

27. A polynucleotide of claim 26 wherein the polypeptide further lacks cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2.

28. A purified polynucleotide comprising a nucleotide sequecne encoding a polypeptide that comprises a fragment of a hu-Asp1 protein, wherein said nucleotide sequence lacks the sequence that encodes amino acids 1-62 of SEQ ID NO: 2, and wherein the polypeptide has hu-Asp1 α-secretase activity.

29. A polypeptide of claim 28, wherein said polypeptide lacks amino terminal amino acids 23-62 of SEQ ID NO: 2.

30. A purified polynucleotide comprising a nucleotide sequence that hybridizes under stringent conditions to the non-coding strand complementary to SEQ ID NO: 1, wherein the nucleotide sequence encodes a polypeptide having Asp1 proteolytic activity and wherein the polynucleotide lacks nucleotides encoding a transmembrane domain.

31. A purified polynucleotide comprising a nucleotide sequence that hybridizes under stringent conditions to the nucleic acid according to claim 30, wherein the nucleotide sequence encodes a polypeptide further lacking a pro-peptide domain corresponding to amino acids 23-62 of SEQ ID NO: 2.

32. A vector comprising the polynucleotide of any one of claims 25-31.

33. A host cell transformed or transfected with a vector of claim 32.

34 A host cell transformed or transfected with a polynucleotide of any one of claims 25-31.

35. A purified polypeptide comprising a fragment of a hu-Asp1 protein, wherein said polypeptide lacks the hu-Asp1 transmembrane domain of said hu-Asp1 protein and retains hu-Asp1 α-secretase activity.

36. A polypeptide of claim 35, wherein said polypeptide comprises a fragment of hu-Asp1 having the amino acid sequence set forth in SEQ ID NO: 2 and wherein said polypeptide lacks the transmembrane domain amino acids 469-492 of SEQ ID NO: 2.

37. A polypeptide according to claim 35 or 36, wherein said polypeptide further lacks cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2.

38. A polypeptide according to any one of claims 35-37, which further lacks amino terminal amino acids 1-62 of SEQ ID NO: 2.

39. A polypeptide comprising a fragment of hu-Asp1 having the amino acid sequence set forth in SEQ ID NO: 2 and wherein said polypeptide lacks the amino terminal amino acids 1-62 of SEQ ID NO: 2 and retains APP processing activity.

40. A polypeptide comprising an amino acid sequence at least 95% identical to a fragment of hu-Asp1 protein, wherein said polypeptide and said fragment lack a transmemebrane domain and retain hu-Asp1 α-secretase activity.

41. A polypeptide comprising an amino acid sequence at least 95% identical to a fragment of hu-Asp1 protein, wherein said polypeptide and said fragment lack the amino terminal amino acids corresponding to the pro-peptide domain of hu-Asp1 and retain APP processing activity.

FIGURE 1 (1)

```
ATGGGCGCACTGGCCCGGGCGCTGCTGCTGCCTCTGCTGGCCCAGTGGCTCCTGCGCGCC
M  G  A  L  A  R  A  L  L  L  P  L  L  A  Q  W  L  L  R  A

CCCCGGAGCTGGCCCCCGCGCCCTTCACGCTGCCCCTCCGGGTGGCCGCGGCCACGAAC
A  P  E  L  A  P  A  P  F  T  L  P  L  R  V  A  A  A  T  N

CGCGTAGTTGCGCCCACCCCGGGACCCGGGACCCCTGCCGAGCGCCACGCCGACGGCTTG
R  V  V  A  P  T  P  G  P  G  T  P  A  E  R  H  A  D  G  L

GCGCTCGCCCTGGAGCCTGCCCTGGCGTCCCCCGCGGGCGCCGCCAACTTCTTGGCCATG
A  L  A  L  E  P  A  L  A  S  P  A  G  A  A  N  F  L  A  M

GTAGACAACCTGCAGGGGGACTCTGGCCGCGGCTACTACCTGGAGATGCTGATCGGGACC
V  D  N  L  Q  G  D  S  G  R  G  Y  Y  L  E  M  L  I  G  T

CCCCCGCAGAAGCTACAGATTCTCGTTGACACTGGAAGCAGTAACTTTGCCGTGGCAGGA
P  P  Q  K  L  Q  I  L  V  D  T  G  S  S  N  F  A  V  A  G

ACCCCGCACTCCTACATAGACACGTACTTTGACACAGAGAGGTCTAGCACATACCGCTCC
T  P  H  S  Y  I  D  T  Y  F  D  T  E  R  S  S  T  Y  R  S

AAGGGCTTTGACGTCACAGTGAAGTACACACAAGGAAGCTGGACGGGCTTCGTTGGGGAA
K  G  F  D  V  T  V  K  Y  T  Q  G  S  W  T  G  F  V  G  E

GACCTCGTCACCATCCCCAAAGGCTTCAATACTTCTTTTCTTGTCAACATTGCCACTATT
D  L  V  T  I  P  K  G  F  N  T  S  F  L  V  N  I  A  T  I

TTTGAATCAGAGAATTTCTTTTTGCCTGGGATTAAATGGAATGGAATACTTGGCCTAGCT
F  E  S  E  N  F  F  L  P  G  I  K  W  N  G  I  L  G  L  A

TATGCCACACTTGCCAAGCCATCAAGTTCTCTGGAGACCTTCTTCGACTCCCTGGTGACA
Y  A  T  L  A  K  P  S  S  S  L  E  T  F  F  D  S  L  V  T

CAAGCAAACATCCCCAACGTTTTCTCCATGCAGATGTGTGGAGCCGGCTTGCCCGTTGCT
Q  A  N  I  P  N  V  F  S  M  Q  M  C  G  A  G  L  P  V  A

GGATCTGGGACCAACGGAGGTAGTCTTGTCTTGGGTGGAATTGAACCAAGTTTGTATAAA
G  S  G  T  N  G  G  S  L  V  L  G  G  I  E  P  S  L  Y  K

GGAGACATCTGGTATACCCCTATTAAGGAAGAGTGGTACTACCAGATAGAAATTCTGAAA
G  D  I  W  Y  T  P  I  K  E  E  W  Y  Y  Q  I  E  I  L  K

TTGGAAATTGGAGGCCAAAGCCTTAATCTGGACTGCAGAGAGTATAACGCAGACAAGGCC
L  E  I  G  G  Q  S  L  N  L  D  C  R  E  Y  N  A  D  K  A

ATCGTGGACAGTGGCACCACGCTGCTGCGCCTGCCCCAGAAGGTGTTTGATGCGGTGGTG
I  V  D  S  G  T  T  L  L  R  L  P  Q  K  V  F  D  A  V  V

GAAGCTGTGGCCCGCGCATCTCTGATTCCAGAATTCTCTGATGGTTTCTGGACTGGGTCC
E  A  V  A  R  A  S  L  I  P  E  F  S  D  G  F  W  T  G  S

CAGCTGGCGTGCTGGACGAATTCGGAAACACCTTGGTCTTACTTCCCTAAAATCTCCATC
Q  L  A  C  W  T  N  S  E  T  P  W  S  Y  F  P  K  I  S  I

TACCTGAGAGATGAGAACTCCAGCAGGTCATTCCGTATCACAATCCTGCCTCAGCTTTAC
Y  L  R  D  E  N  S  S  R  S  F  R  I  T  I  L  P  Q  L  Y

ATTCAGCCCATGATGGGGGCCGGCCTGAATTATGAATGTTACCGATTCGGCATTTCCCCA
I  Q  P  M  M  G  A  G  L  N  Y  E  C  Y  R  F  G  I  S  P
```

```
TCCACAAATGCGCTGGTGATCGGTGCCACGGTGATGGAGGGCTTCTACGTCATCTTCGAC
S  T  N  A  L  V  I  G  A  T  V  M  E  G  F  Y  V  I  F  D

AGAGCCCAGAAGAGGGTGGGCTTCGCAGCGAGCCCCTGTGCAGAAATTGCAGGTGCTGCA
```

FIGURE 1 (2)

R A Q K R V G F A A S P C A E I A G A A

GTGTCTGAAATTTCCGGGCCTTTCTCAACAGAGGATGTAGCCAGCAACTGTGTCCCCGCT
V S E I S G P F S T E D V A S N C V P A

CAGTCTTTGAGCGAGCCCATTTTGTGGATTGTGTCCTATGCGCTCATGAGCGTCTGTGGA
Q S L S E P I L W I V S Y A L M S V C G

GCCATCCTCCTTGTCTTAATCGTCCTGCTGCTGCTGCCGTTCCGGTGTCAGCGTCGCCCC
A I L L V L I V L L L L P F R C Q R R P

CGTGACCCTGAGGTCGTCAATGATGAGTCCTCTCTGGTCAGACATCGCTGGAAATGAATA
R D P E V V N D E S S L V R H R W K

GCCAGGCCTGACCTCAAGCAACCATGAACTCAGCTATTAAGAAAATCACATTTCCAGGGC
AGCAGCCGGGATCGATGGTGGCGCTTTCTCCTGTGCCCACCCGTCTTCAATCTCTGTTCT
GCTCCCAGATGCCTTCTAGATTCACTGTCTTTTGATTCTTGATTTTCAAGCTTTCAAATC
CTCCCTACTTCCAAGAAAAATAATTAAAAAAAAAACTTCATTCTAAACCAAAAAAAAAAA
AAAA

FIGURE 2 (1)

ATGGCCCAAGCCCTGCCCTGGCTCCTGCTGTGGATGGGCGCGGGAGTGCTGCCTGCCCAC
M A Q A L P W L L L W M G A G V L P A H

GGCACCCAGCACGGCATCCGGCTGCCCCTGCGCAGCGGCCTGGGGGCGCCCCCCTGGGG
G T Q H G I R L P L R S G L G G A P L G

CTGCGGCTGCCCCGGGAGACCGACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTT
L R L P R E T D E E P E E P G R R G S F

GTGGAGATGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACC
V E M V D N L R G K S G Q G Y Y V E M T

GTGGGCAGCCCCCCGCAGACGCTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCA
V G S P P Q T L N I L V D T G S S N F A

GTGGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACA
V G A A P H P F L H R Y Y Q R Q L S S T

TACCGGGACCTCCGGAAGGGTGTGTATGTGCCCTACACCCAGGGCAAGTGGGAAGGGGAG
Y R D L R K G V Y V P Y T Q G K W E G E

CTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTCACTGTGCGTGCCAACATT
L G T D L V S I P H G P N V T V R A N I

GCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCCAACTGGGAAGGCATCCTG
A A I T E S D K F F I N G S N W E G I L

GGGCTGGCCTATGCTGAGATTGCCAGGCTTTGTGGTGCTGGCTTCCCCCTCAACCAGTCT
G L A Y A E I A R L C G A G F P L N Q S

GAAGTGCTGGCCTCTGTCGGAGGGAGCATGATCATTGGAGGTATCGACCACTCGCTGTAC
E V L A S V G G S M I I G G I D H S L Y

ACAGGCAGTCTCTGGTATACACCCATCCGGCGGGAGTGGTATTATGAGGTGATCATTGTG
T G S L W Y T P I R R E W Y Y E V I I V

CGGGTGGAGATCAATGGACAGGATCTGAAAATGGACTGCAAGGAGTACAACTATGACAAG
R V E I N G Q D L K M D C K E Y N Y D K

AGCATTGTGGACAGTGGCACCACCAACCTTCGTTTGCCCAAGAAAGTGTTTGAAGCTGCA
S I V D S G T T N L R L P K K V F E A A

GTCAAATCCATCAAGGCAGCCTCCTCCACGGAGAAGTTCCCTGATGGTTTCTGGCTAGGA
V K S I K A A S S T E K F P D G F W L G

GAGCAGCTGGTGTGCTGGCAAGCAGGCACCACCCCTTGGAACATTTTCCCAGTCATCTCA
E Q L V C W Q A G T T P W N I F P V I S

CTCTACCTAATGGGTGAGGTTACCAACCAGTCCTTCCGCATCACCATCCTTCCGCAGCAA
L Y L M G E V T N Q S F R I T I L P Q Q

TACCTGCGGCCAGTGGAAGATGTGGCCACGTCCCAAGACGACTGTTACAAGTTTGCCATC

FIGURE 2 (2)

Y L R P V E D V A T S Q D D C Y K F A I

TCACAGTCATCCACGGGCACTGTTATGGGAGCTGTTATCATGGAGGGCTTCTACGTTGTC
S Q S S T G T V M G A V I M E G F Y V V

TTTGATCGGGCCCGAAAACGAATTGGCTTTGCTGTCAGCGCTTGCCATGTGCACGATGAG
F D R A R K R I G F A V S A C H V H D E

TTCAGGACGGCAGCGGTGGAAGGCCCTTTTGTCACCTTGGACATGGAAGACTGTGGCTAC
F R T A A V E G P F V T L D M E D C G Y

AACATTCCACAGACAGATGAGTCAACCCTCATGACCATAGCCTATGTCATGGCTGCCATC
N I P Q T D E S T L M T I A Y V M A A I

TGCGCCCTCTTCATGCTGCCACTCTGCCTCATGGTGTGTCAGTGGCGCTGCCTCCGCTGC
C A L F M L P L C L M V C Q W R C L R C

CTGCGCCAGCAGCATGATGACTTTGCTGATGACATCTCCCTGCTGAAGTGAGGAGGCCCA
L R Q Q H D D F A D D I S L L K

TGGGCAGAAGATAGAGATTCCCCTGGACCACACCTCCGTGGTTCACTTTGGTCACAAGTA
GGAGACACAGATGGCACCTGTGGCCAGAGCACCTCAGGACCCTCCCCACCCACCAAATGC
CTCTGCCTTGATGGAGAAGGAAAAGGCTGGCAAGGTGGGTTCCAGGGACTGTACCTGTAG
GAAACAGAAAAGAGAAGAAAGAAGCACTCTGCTGGCGGGAATACTCTTGGTCACCTCAAA
TTTAAGTCGGGAAATTCTGCTGCTTGAAACTTCAGCCCTGAACCTTTGTCCACCATTCCT
TTAAATTCTCCAACCCAAAGTATTCTTCTTTTCTTAGTTTCAGAAGTACTGGCATCACAC
GCAGGTTACCTTGGCGTGTGTCCCTGTGGTACCCTGGCAGAGAAGAGACCAAGCTTGTTT
CCCTGCTGGCCAAAGTCAGTAGGAGAGGATGCACAGTTTGCTATTTGCTTTAGAGACAGG
GACTGTATAAACAAGCCTAACATTGGTGCAAAGATTGCCTCTTGAAAAAAAAAAAAA

FIGURE 3 (1)

```
ATGGCCCAAGCCCTGCCCTGGCTCCTGCTGTGGATGGGCGCGGGAGTGCTGCCTGCCCAC
M  A  Q  A  L  P  W  L  L  L  W  M  G  A  G  V  L  P  A  H

GGCACCCAGCACGGCATCCGGCTGCCCCTGCGCAGCGGCCTGGGGGGCGCCCCCCTGGGG
G  T  Q  H  G  I  R  L  P  L  R  S  G  L  G  G  A  P  L  G

CTGCGGCTGCCCCGGGAGACCGACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTT
L  R  L  P  R  E  T  D  E  E  P  E  E  P  G  R  R  G  S  F

GTGGAGATGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACC
V  E  M  V  D  N  L  R  G  K  S  G  Q  G  Y  Y  V  E  M  T

GTGGGCAGCCCCCCGCAGACGCTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCA
V  G  S  P  P  Q  T  L  N  I  L  V  D  T  G  S  S  N  F  A

GTGGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACA
V  G  A  A  P  H  P  F  L  H  R  Y  Y  Q  R  Q  L  S  S  T

TACCGGGACCTCCGGAAGGGTGTGTATGTGCCCTACACCCAGGGCAAGTGGGAAGGGGAG
Y  R  D  L  R  K  G  V  Y  V  P  Y  T  Q  G  K  W  E  G  E

CTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTCACTGTGCGTGCCAACATT
L  G  T  D  L  V  S  I  P  H  G  P  N  V  T  V  R  A  N  I

GCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCCAACTGGGAAGGCATCCTG
A  A  I  T  E  S  D  K  F  F  I  N  G  S  N  W  E  G  I  L

GGGCTGGCCTATGCTGAGATTGCCAGGCCTGACGACTCCCTGGAGCCTTTCTTTGACTCT
G  L  A  Y  A  E  I  A  R  P  D  D  S  L  E  P  F  F  D  S

CTGGTAAAGCAGACCCACGTTCCCAACCTCTTCTCCCTGCAGCTTTGTGGTGCTGGCTTC
L  V  K  Q  T  H  V  P  N  L  F  S  L  Q  L  C  G  A  G  F

CCCCTCAACCAGTCTGAAGTGCTGGCCTCTGTCGGAGGGAGCATGATCATTGGAGGTATC
P  L  N  Q  S  E  V  L  A  S  V  G  G  S  M  I  I  G  G  I

GACCACTCGCTGTACACAGGCAGTCTCTGGTATACACCCATCCGGCGGGAGTGGTATTAT
D  H  S  L  Y  T  G  S  L  W  Y  T  P  I  R  R  E  W  Y  Y

GAGGTCATCATTGTGCGGGTGGAGATCAATGGACAGGATCTGAAAATGGACTGCAAGGAG
E  V  I  I  V  R  V  E  I  N  G  Q  D  L  K  M  D  C  K  E

TACAACTATGACAAGAGCATTGTGGACAGTGGCACCACCAACCTTCGTTTGCCCAAGAAA
Y  N  Y  D  K  S  I  V  D  S  G  T  T  N  L  R  L  P  K  K

GTGTTTGAAGCTGCAGTCAAATCCATCAAGGCAGCCTCCTCCACGGAGAAGTTCCCTGAT
V  F  E  A  A  V  K  S  I  K  A  A  S  S  T  E  K  F  P  D
```

FIGURE 3 (2)

```
GGTTTCTGGCTAGGAGAGCAGCTGGTGTGCTGGCAAGCAGGCACCACCCCTTGGAACATT
G  F  W  L  G  E  Q  L  V  C  W  Q  A  G  T  T  P  W  N  I

TTCCCAGTCATCTCACTCTACCTAATGGGTGAGGTTACCAACCAGTCCTTCCGCATCACC
F  P  V  I  S  L  Y  L  M  G  E  V  T  N  Q  S  F  R  I  T

ATCCTTCCGCAGCAATACCTGCGGCCAGTGGAAGATGTGGCCACGTCCCAAGACGACTGT
I  L  P  Q  Q  Y  L  R  P  V  E  D  V  A  T  S  Q  D  D  C

TACAAGTTTGCCATCTCACAGTCATCCACGGGCACTGTTATGGGAGCTGTTATCATGGAG
Y  K  F  A  I  S  Q  S  S  T  G  T  V  M  G  A  V  I  M  E

GGCTTCTACGTTGTCTTTGATCGGGCCCGAAAACGAATTGGCTTTGCTGTCAGCGCTTGC
G  F  Y  V  V  F  D  R  A  R  K  R  I  G  F  A  V  S  A  C

CATGTGCACGATGAGTTCAGGACGGCAGCGGTGGAAGGCCCTTTTGTCACCTTGGACATG
H  V  H  D  E  F  R  T  A  A  V  E  G  P  F  V  T  L  D  M

GAAGACTGTGGCTACAACATTCCACAGACAGATGAGTCAACCCTCATGACCATAGCCTAT
E  D  C  G  Y  N  I  P  Q  T  D  E  S  T  L  M  T  I  A  Y

GTCATGGCTGCCATCTGCGCCCTCTTCATGCTGCCACTCTGCCTCATGGTGTGTCAGTGG
V  M  A  A  I  C  A  L  F  M  L  P  L  C  L  M  V  C  Q  W

CGCTGCCTCCGCTGCCTGCGCCAGCAGCATGATGACTTTGCTGATGACATCTCCCTGCTG
R  C  L  R  C  L  R  Q  Q  H  D  D  F  A  D  D  I  S  L  L

AAGTGAGGAGGCCCATGGGCAGAAGATAGAGATTCCCCTGGACCACACCTCCGTGGTTCA
K

CTTTGGTCACAAGTAGGAGACACAGATGGCACCTGTGGCCAGAGCACCTCAGGACCCTCC
CCACCCACCAAATGCCTCTGCCTTGATGGAGAAGGAAAAGGCTGGCAAGGTGGGTTCCAG
GGACTGTACCTGTAGGAAACAGAAAAGAGAAGAAAGAAGCACTCTGCTGGCGGGAATACT
CTTGGTCACCTCAAATTTAAGTCGGGAAATTCTGCTGCTTGAAACTTCAGCCCTGAACCT
TTGTCCACCATTCCTTTAAATTCTCCAACCCAAAGTATTCTTCTTTTCTTAGTTTCAGAA
GTACTGGCATCACACGCAGGTTACCTTGGCGTGTGTCCCTGTGGTACCCTGGCAGAGAAG
AGACCAAGCTTGTTTCCCTGCTGGCCAAAGTCAGTAGGAGAGGATGCACAGTTTGCTATT
TGCTTTAGAGACAGGGACTGTATAAACAAGCCTAACATTGGTGCAAAGATTGCCTCTTGA
ATTAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

FIGURE 4

ATGGCCCCAGCGCTGCACTGGCTCCTGCTATGGGTGGGCTCGGGAATGCTGCCTGCCCAG
M A P A L H W L L L W V G S G M L P A Q
GGAACCCATCTCGGCATCCGGCTGCCCCTTCGCAGCGGCCTGGCAGGGCCACCCCTGGGC
G T H L G I R L P L R S G L A G P P L G
CTGAGGCTGCCCCGGGAGACTGACGAGGAATCGGAGGAGCCTGGCCGGAGAGGCAGCTTT
L R L P R E T D E E S E E P G R R G S F
GTGGAGATGGTGGACAACCTGAGGGGAAAGTCCGGCCAGGGCTACTATGTGGAGATGACC
V E M V D N L R G K S G Q G Y Y V E M T
GTAGGCAGCCCCCCACAGACGCTCAACATCCTGGTGGACACGGGCAGTAGTAACTTTGCA
V G S P P Q T L N I L V D T G S S N F A
GTGGGGGCTGCCCCACACCCTTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACA
V G A A P H P F L H R Y Y Q R Q L S S T
TATCGAGACCTCCGAAAGGGTGTGTATGTGCCCTACACCCAGGGCAAGTGGGAGGGGGAA
Y R D L R K G V Y V P Y T Q G K W E G E
CTGGGCACCGACCTGGTGAGCATCCCTCATGGCCCCAACGTCACTGTGCGTGCCAACATT
L G T D L V S I P H G P N V T V R A N I
GCTGCCATCACTGAATCGGACAAGTTCTTCATCAATGGTTCCAACTGGGAGGGCATCCTA
A A I T E S D K F F I N G S N W E G I L
GGGCTGGCCTATGCTGAGATTGCCAGGCCCGACGACTCTTTGGAGCCCTTCTTTGACTCC
G L A Y A E I A R P D D S L E P F F D S
CTGGTGAAGCAGACCCACATTCCCAACATCTTTTCCCTGCAGCTCTGTGGCGCTGGCTTC
L V K Q T H I P N I F S L Q L C G A G F
CCCCTCAACCAGACCGAGGCACTGGCCTCGGTGGGAGGGAGCATGATCATTGGTGGTATC
P L N Q T E A L A S V G G S M I I G G I
GACCACTCGCTATACACGGGCAGTCTCTGGTACACACCCATCCGGCGGGAGTGGTATTAT
D H S L Y T G S L W Y T P I R R E W Y Y
GAAGTGATCATTGTACGTGTGGAAATCAATGGTCAAGATCTCAAGATGGACTGCAAGGAG
E V I I V R V E I N G Q D L K M D C K E
TACAACTACGACAAGAGCATTGTGGACAGTGGGACCACCAACCTTCGCTTGCCCAAGAAA
Y N Y D K S I V D S G T T N L R L P K K
GTATTTGAAGCTGCCGTCAAGTCCATCAAGGCAGCCTCCTCGACGGAGAAGTTCCCGGAT
V F E A A V K S I K A A S S T E K F P D
GGCTTTTGGCTAGGGGAGCAGCTGGTGTGCTGGCAAGCAGGCACGACCCCTTGGAACATT
G F W L G E Q L V C W Q A G T T P W N I
TTCCCAGTCATTTCACTTTACCTCATGGGTGAAGTCACCAATCAGTCCTTCCGCATCACC
F P V I S L Y L M G E V T N Q S F R I T
ATCCTTCCTCAGCAATACCTACGGCCGGTGGAGGACGTGGCCACGTCCCAAGACGACTGT
I L P Q Q Y L R P V E D V A T S Q D D C
TACAAGTTCGCTGTCTCACAGTCATCCACGGGCACTGTTATGGGAGCCGTCATCATGGAA
Y K F A V S Q S S T G T V M G A V I M E
GGTTTCTATGTCGTCTTCGATCGAGCCCGAAAGCGAATTGGCTTTGCTGTCAGCGCTTGC
G F Y V V F D R A R K R I G F A V S A C
CATGTGCACGATGAGTTCAGGACGGCGGCAGTGGAAGGTCCGTTTGTTACGGCAGACATG
H V H D E F R T A A V E G P F V T A D M
GAAGACTGTGGCTACAACATTCCCCAGACAGATGAGTCAACACTTATGACCATAGCCTAT
E D C G Y N I P Q T D E S T L M T I A Y
GTCATGGCGGCCATCTGCGCCCTCTTCATGTTGCCACTCTGCCTCATGGTATGTCAGTGG
V M A A I C A L F M L P L C L M V C Q W
CGCTGCCTGCGTTGCCTGCGCCACCAGCACGATGACTTTGCTGATGACATCTCCCTGCTC
R C L R C L R H Q H D D F A D D I S L L
AAGTAAGGAGGCTCGTGGGCAGATGATGGAGACGCCCCTGGACCACATCTGGGTGGTTCC
K
CTTTGGTCACATGAGTTGGAGCTATGGATGGTACCTGTGGCCAGAGCACCTCAGGACCCT
CACCAACCTGCCAATGCTTCTGGCGTGACAGAACAGAGAAATCAGGCAAGCTGGATTACA
GGGCTTGCACCTGTAGGACACAGGAGAGGGAAGGAAGCAGCGTTCTGGTGGCAGGAATAT
CCTTAGGCACCACAAACTTGAGTTGGAAATTTTGCTGCTTGAAGCTTCAGCCCTGACCCT
CTGCCCAGCATCCTTTAGAGTCTCCAACCTAAAGTATTCTTTATGTCCTTCCAGAAGTAC

TGGCGTCATACTCAGGCTACCCGGCATGTGTCCCTGTGGTACCCTGGCAGAGAAAGGGCC
AATCTCATTCCCTGCTGGCCAAAGTCAGCAGAAGAAGGTGAAGTTTGCCAGTTGCTTTAG
TGATAGGGACTGCAGACTCAAGCCTACACTGGTACAAAGACTGCGTCTTGAGATAAACAA
GAA

FIGURE 5

```
  1 MAQALPWLLLWMGAGVLPAHGTQHGIRLPLRSGLGGAPLGLRLPRETDEE 50
    || || |||||.|.|.||| ||  |||||||||| | |||||||||||||
  1 MAPALHWLLLWVGSGMLPAQGTHLGIRLPLRSGLAGPPLGLRLPRETDEE 50
             .         .         .         .         .
 51 PEEPGRRGSFVEMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFA 100
     |||||||||||||||||||||||||||||||||||||||||||||||||
 51 SEEPGRRGSFVEMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFA 100
             .         .         .         .         .
101 VGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPYTQGKWEGELGTDLVSIPH 150
    ||||||||||||||||||||||||||||||||||||||||||||||||||
101 VGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPYTQGKWEGELGTDLVSIPH 150
             .         .         .         .         .
151 GPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDS 200
    ||||||||||||||||||||||||||||||||||||||||||||||||||
151 GPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDS 200
             .         .         .         .         .
201 LVKQTHVPNLFSLQLCGAGFPLNQSEVLASVGGSMIIGGIDHSLYTGSLW 250
    ||||||:||:||||||||||||||.| |||||||||||||||||||||||
201 LVKQTHIPNIFSLQLCGAGFPLNQTEALASVGGSMIIGGIDHSLYTGSLW 250
             .         .         .         .         .
251 YTPIRREWYYEVIIVRVEINGQDLKMDCKEYNYDKSIVDSGTTNLRLPKK 300
    ||||||||||||||||||||||||||||||||||||||||||||||||||
251 YTPIRREWYYEVIIVRVEINGQDLKMDCKEYNYDKSIVDSGTTNLRLPKK 300
             .         .         .         .         .
301 VFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIFPVISLYLMG 350
    ||||||||||||||||||||||||||||||||||||||||||||||||||
301 VFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIFPVISLYLMG 350
             .         .         .         .         .
351 EVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAISQSSTGTVMGAVIME 400
    |||||||||||||||||||||||||||||||||||:||||||||||||||
351 EVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAVSQSSTGTVMGAVIME 400
             .         .         .         .         .
401 GFYVVFDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQT 450
    ||||||||||||||||||||||||||||||| ||||||||||||||||||
401 GFYVVFDRARKRIGFAVSACHVHDEFRTAAVEGPFVTADMEDCGYNIPQT 450
             .         .         .         .         .
451 DESTLMTIAYVMAAICALFMLPLCLMVCQWRCLRCLRQQHDDFADDISLL 500
    |||||||||||||||||||||||||||||||| |||||||||||||||||
451 DESTLMTIAYVMAAICALFMLPLCLMVCQWRCLRCLRHQHDDFADDISLL 500

501 K 501
    |
501 K 501
```

FIGURE 6 (1)

```
ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCACCCAGCACGGCATCCGG
M  A  S  M  T  G  G  Q  Q  M  G  R  G  S  T  Q  H  G  I  R

CTGCCCCTGCGCAGCGGCCTGGGGGGCGCCCCCCTGGGGCTGCGGCTGCCCCGGGAGACC
L  P  L  R  S  G  L  G  G  A  P  L  G  L  R  L  P  R  E  T

GACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTTGTGGAGATGGTGGACAACCTG
D  E  E  P  E  E  P  G  R  R  G  S  F  V  E  M  V  D  N  L

AGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACCGTGGGCAGCCCCCCGCAGACG
R  G  K  S  G  Q  G  Y  Y  V  E  M  T  V  G  S  P  P  Q  T

CTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCAGTGGGTGCTGCCCCCCACCCC
L  N  I  L  V  D  T  G  S  S  N  F  A  V  G  A  A  P  H  P

TTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACATACCGGGACCTCCGGAAGGGC
F  L  H  R  Y  Y  Q  R  Q  L  S  S  T  Y  R  D  L  R  K  G

GTGTATGTGCCCTACACCCAGGGCAAGTGGGAAGGGGAGCTGGGCACCGACCTGGTAAGC
V  Y  V  P  Y  T  Q  G  K  W  E  G  E  L  G  T  D  L  V  S

ATCCCCCATGGCCCCAACGTCACTGTGCGTGCCAACATTGCTGCCATCACTGAATCAGAC
I  P  H  G  P  N  V  T  V  R  A  N  I  A  A  I  T  E  S  D

AAGTTCTTCATCAACGGCTCCAACTGGGAAGGCATCCTGGGGCTGGCCTATGCTGAGATT
K  F  F  I  N  G  S  N  W  E  G  I  L  G  L  A  Y  A  E  I

GCCAGGCCTGACGACTCCCTGGAGCCTTTCTTTGACTCTCTGGTAAAGCAGACCCACGTT
A  R  P  D  D  S  L  E  P  F  F  D  S  L  V  K  Q  T  H  V

CCCAACCTCTTCTCCCTGCAGCTTTGTGGTGCTGGCTTCCCCCTCAACCAGTCTGAAGTG
P  N  L  F  S  L  Q  L  C  G  A  G  F  P  L  N  Q  S  E  V

CTGGCCTCTGTCGGAGGGAGCATGATCATTGGAGGTATCGACCACTCGCTGTACACAGGC
L  A  S  V  G  G  S  M  I  I  G  G  I  D  H  S  L  Y  T  G

AGTCTCTGGTATACACCCATCCGGCGGGAGTGGTATTATGAGGTCATCATTGTGCGGGTG
S  L  W  Y  T  P  I  R  R  E  W  Y  Y  E  V  I  I  V  R  V

GAGATCAATGGACAGGATCTGAAAATGGACTGCAAGGAGTACAACTATGACAAGAGCATT
E  I  N  G  Q  D  L  K  M  D  C  K  E  Y  N  Y  D  K  S  I

GTGGACAGTGGCACCACCAACCTTCGTTTGCCCAAGAAAGTGTTTGAAGCTGCAGTCAAA
V  D  S  G  T  T  N  L  R  L  P  K  K  V  F  E  A  A  V  K

TCCATCAAGGCAGCCTCCTCCACGGAGAAGTTCCCTGATGGTTTCTGGCTAGGAGAGCAG
S  I  K  A  A  S  S  T  E  K  F  P  D  G  F  W  L  G  E  Q

CTGGTGTGCTGGCAAGCAGGCACCACCCCTTGGAACATTTTCCCAGTCATCTCACTCTAC
L  V  C  W  Q  A  G  T  T  P  W  N  I  F  P  V  I  S  L  Y

CTAATGGGTGAGGTTACCAACCAGTCCTTCCGCATCACCATCCTTCCGCAGCAATACCTG
L  M  G  E  V  T  N  Q  S  F  R  I  T  I  L  P  Q  Q  Y  L

CGGCCAGTGGAAGATGTGGCCACGTCCCAAGACGACTGTTACAAGTTTGCCATCTCACAG
```

FIGURE 6 (2)

R P V E D V A T S Q D D C Y K F A I S Q

TCATCCACGGGCACTGTTATGGGAGCTGTTATCATGGAGGGCTTCTACGTTGTCTTTGAT
S S T G T V M G A V I M E G F Y V V F D

CGGGCCCGAAAACGAATTGGCTTTGCTGTCAGCGCTTGCCATGTGCACGATGAGTTCAGG
R A R K R I G F A V S A C H V H D E F R

ACGGCAGCGGTGGAAGGCCCTTTTGTCACCTTGGACATGGAAGACTGTGGCTACAACATT
T A A V E G P F V T L D M E D C G Y N I

CCACAGACAGATGAGTCATGA
P Q T D E S *

FIGURE 7 (1)

ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCGATGACTATCTCTGACTCT
M A S M T G G Q Q M G R G S M T I S D S

CCGCGTGAACAGGACGGATCCACCCAGCACGGCATCCGGCTGCCCCTGCGCAGCGGCCTG
P R E Q D G S T Q H G I R L P L R S G L

GGGGGCGCCCCCCTGGGGCTGCGGCTGCCCCGGGAGACCGACGAAGAGCCCGAGGAGCCC
G G A P L G L R L P R E T D E E P E E P

GGCCGGAGGGGCAGCTTTGTGGAGATGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGC
G R R G S F V E M V D N L R G K S G Q G

TACTACGTGGAGATGACCGTGGGCAGCCCCCCGCAGACGCTCAACATCCTGGTGGATACA
Y Y V E M T V G S P P Q T L N I L V D T

GGCAGCAGTAACTTTGCAGTGGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAG
G S S N F A V G A A P H P F L H R Y Y Q

AGGCAGCTGTCCAGCACATACCGGGACCTCCGGAAGGGCGTGTATGTGCCCTACACCCAG
R Q L S S T Y R D L R K G V Y V P Y T Q

GGCAAGTGGGAAGGGGAGCTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTC
G K W E G E L G T D L V S I P H G P N V

ACTGTGCGTGCCAACATTGCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCC
T V R A N I A A I T E S D K F F I N G S

AACTGGGAAGGCATCCTGGGGCTGGCCTATGCTGAGATTGCCAGGCCTGACGACTCCCTG
N W E G I L G L A Y A E I A R P D D S L

GAGCCTTTCTTTGACTCTCTGGTAAAGCAGACCCACGTTCCCAACCTCTTCTCCCTGCAG
E P F F D S L V K Q T H V P N L F S L Q

CTTTGTGGTGCTGGCTTCCCCCTCAACCAGTCTGAAGTGCTGGCCTCTGTCGGAGGGAGC
L C G A G F P L N Q S E V L A S V G G S

ATGATCATTGGAGGTATCGACCACTCGCTGTACACAGGCAGTCTCTGGTATACACCCATC
M I I G G I D H S L Y T G S L W Y T P I

CGGCGGGAGTGGTATTATGAGGTCATCATTGTGCGGGTGGAGATCAATGGACAGGATCTG
R R E W Y Y E V I I V R V E I N G Q D L

AAAATGGACTGCAAGGAGTACAACTATGACAAGAGCATTGTGGACAGTGGCACCACCAAC
K M D C K E Y N Y D K S I V D S G T T N

CTTCGTTTGCCCAAGAAAGTGTTTGAAGCTGCAGTCAAATCCATCAAGGCAGCCTCCTCC
L R L P K K V F E A A V K S I K A A S S

ACGGAGAAGTTCCCTGATGGTTTCTGGCTAGGAGAGCAGCTGGTGTGCTGGCAAGCAGGC
T E K F P D G F W L G E Q L V C W Q A G

ACCACCCCTTGGAACATTTTCCCAGTCATCTCACTCTACCTAATGGGTGAGGTTACCAAC
T T P W N I F P V I S L Y L M G E V T N

FIGURE 7 (2)

```
CAGTCCTTCCGCATCACCATCCTTCCGCAGCAATACCTGCGGCCAGTGGAAGATGTGGCC
Q  S  F  R  I  T  I  L  P  Q  Q  Y  L  R  P  V  E  D  V  A

ACGTCCCAAGACGACTGTTACAAGTTTGCCATCTCACAGTCATCCACGGGCACTGTTATG
T  S  Q  D  D  C  Y  K  F  A  I  S  Q  S  S  T  G  T  V  M

GGAGCTGTTATCATGGAGGGCTTCTACGTTGTCTTTGATCGGGCCCGAAAACGAATTGGC
G  A  V  I  M  E  G  F  Y  V  V  F  D  R  A  R  K  R  I  G

TTTGCTGTCAGCGCTTGCCATGTGCACGATGAGTTCAGGACGGCAGCGGTGGAAGGCCCT
F  A  V  S  A  C  H  V  H  D  E  F  R  T  A  A  V  E  G  P

TTTGTCACCTTGGACATGGAAGACTGTGGCTACAACATTCCACAGACAGATGAGTCATGA
F  V  T  L  D  M  E  D  C  G  Y  N  I  P  Q  T  D  E  S  *
```

FIGURE 8 (1)

```
ATGACTCAGCATGGTATTCGTCTGCCACTGCGTAGCGGTCTGGGTGGTGCTCCACTGGGT
M  T  Q  H  G  I  R  L  P  L  R  S  G  L  G  G  A  P  L  G   -

CTGCGTCTGCCCCGGGAGACCGACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTT
L  R  L  P  R  E  T  D  E  E  P  E  E  P  G  R  R  G  S  F   -

GTGGAGATGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACC
V  E  M  V  D  N  L  R  G  K  S  G  Q  G  Y  Y  V  E  M  T   -

GTGGGCAGCCCCCCGCAGACGCTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCA
V  G  S  P  P  Q  T  L  N  I  L  V  D  T  G  S  S  N  F  A   -

GTGGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACA
V  G  A  A  P  H  P  F  L  H  R  Y  Y  Q  R  Q  L  S  S  T   -

TACCGGGACCTCCGGAAGGGCGTGTATGTGCCCTACACCCAGGGCAAGTGGGAAGGGGAG
Y  R  D  L  R  K  G  V  Y  V  P  Y  T  Q  G  K  W  E  G  E   -

CTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTCACTGTGCGTGCCAACATT
L  G  T  D  L  V  S  I  P  H  G  P  N  V  T  V  R  A  N  I   -

GCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCCAACTGGGAAGGCATCCTG
A  A  I  T  E  S  D  K  F  F  I  N  G  S  N  W  E  G  I  L   -

GGGCTGGCCTATGCTGAGATTGCCAGGCCTGACGACTCCCTGGAGCCTTTCTTTGACTCT
G  L  A  Y  A  E  I  A  R  P  D  D  S  L  E  P  F  F  D  S

CTGGTAAAGCAGACCCACGTTCCCAACCTCTTCTCCCTGCAGCTTTGTGGTGCTGGCTTC
L  V  K  Q  T  H  V  P  N  L  F  S  L  Q  L  C  G  A  G  F   -

CCCCTCAACCAGTCTGAAGTGCTGGCCTCTGTCGGAGGGAGCATGATCATTGGAGGTATC
P  L  N  Q  S  E  V  L  A  S  V  G  G  S  M  I  I  G  G  I   -

GACCACTCGCTGTACACAGGCAGTCTCTGGTATACACCCATCCGGCGGGAGTGGTATTAT
D  H  S  L  Y  T  G  S  L  W  Y  T  P  I  R  R  E  W  Y  Y   -

GAGGTCATCATTGTGCGGGTGGAGATCAATGGACAGGATCTGAAAATGGACTGCAAGGAG
E  V  I  I  V  R  V  E  I  N  G  Q  D  L  K  M  D  C  K  E

TACAACTATGACAAGAGCATTGTGGACAGTGGCACCACCAACCTTCGTTTGCCCAAGAAA
Y  N  Y  D  K  S  I  V  D  S  G  T  T  N  L  R  L  P  K  K   -

GTGTTTGAAGCTGCAGTCAAATCCATCAAGGCAGCCTCCTCCACGGAGAAGTTCCCTGAT
V  F  E  A  A  V  K  S  I  K  A  A  S  S  T  E  K  F  P  D   -

GGTTTCTGGCTAGGAGAGCAGCTGGTGTGCTGGCAAGCAGGCACCACCCCTTGGAACATT
G  F  W  L  G  E  Q  L  V  C  W  Q  A  G  T  T  P  W  N  I   -

TTCCCAGTCATCTCACTCTACCTAATGGGTGAGGTTACCAACCAGTCCTTTCGCATCACC
F  P  V  I  S  L  Y  L  M  G  E  V  T  N  Q  S  F  R  I  T   -

ATCCTTCCGCAGCAATACCTGCGGCCAGTGGAAGATGTGGCCACGTCCCAAGACGACTGT
I  L  P  Q  Q  Y  L  R  P  V  E  D  V  A  T  S  Q  D  D  C   -
```

FIGURE 8 (2)

TACAAGTTTGCCATCTCACAGTCATCCACGGGCACTGTTATGGGAGCTGTTATCATGGAG
Y K F A I S Q S S T G T V M G A V I M E -

GGCTTCTACGTTGTCTTTGATCGGGCCCGAAAACGAATTGGCTTTGCTGTCAGCGCTTGC
G F Y V V F D R A R K R I G F A V S A C -

CATTAG
H *

FIGURE 9

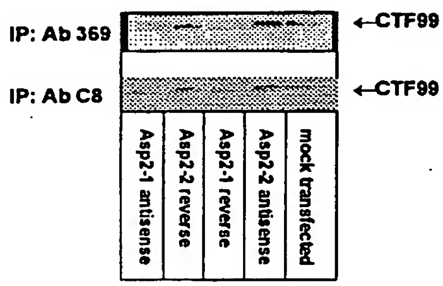
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FIGURE 10

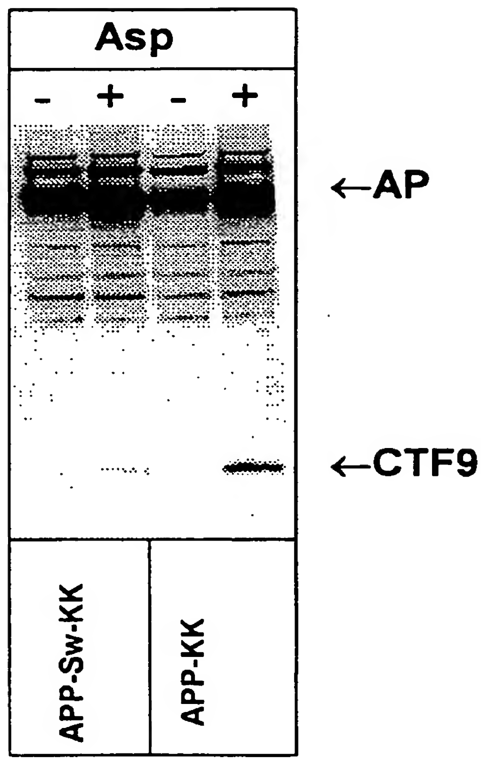

FIGURE 11

<u>MAQALPWLLLWMGAGVLPAHG</u>TQHGIRLPLRSGLGGAPLGLRLPRETDEE
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VGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPYTQGKWEGELGTDLVSIPH
GPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDS
LVKQTHVPNLFSLQLCGAGFPLNQSEVLASVGGSMIIGGIDHSLYTGSLW
YTPIRREWYYEVIIVRVEINGQDLKMDCKEYNYDKSIVDSGTTNLRLPKK
VFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIFPVISLYLMG
EVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAISQSSTGTVMGAVIME
GFYVVFDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQT
DES

FIGURE 12

MAQALPWLLLWMGAGVLPAHGTQHGIRLPLRSGLGGAPLGLRLPRETDEE
PEEPGRRGSFVEMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFA
VGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPYTQGKWEGELGTDLVSIPH
GPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDS
LVKQTHVPNLFSLQLCGAGFPLNQSEVLASVGGSMIIGGIDHSLYTGSLW
YTPIRREWYYEVIIVRVEINGQDLKMDCKEYNYDKSIVDSGTTNLRLPKK
VFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIFPVISLYLMG
EVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAISQSSTGTVMGAVIME
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Leu Leu Arg Ala Ala Pro Glu Leu Ala Pro Ala Pro Phe Thr Leu Pro
             20                  25                  30

Leu Arg Val Ala Ala Ala Thr Asn Arg Val Val Ala Pro Thr Pro Gly
         35                  40                  45

Pro Gly Thr Pro Ala Glu Arg His Ala Asp Gly Leu Ala Leu Ala Leu
     50                  55                  60

Glu Pro Ala Leu Ala Ser Pro Ala Gly Ala Ala Asn Phe Leu Ala Met
 65                  70                  75                  80

Val Asp Asn Leu Gln Gly Asp Ser Gly Arg Gly Tyr Tyr Leu Glu Met
                 85                  90                  95

Leu Ile Gly Thr Pro Pro Gln Lys Leu Gln Ile Leu Val Asp Thr Gly
            100                 105                 110

Ser Ser Asn Phe Ala Val Ala Gly Thr Pro His Ser Tyr Ile Asp Thr
        115                 120                 125

Tyr Phe Asp Thr Glu Arg Ser Ser Thr Tyr Arg Ser Lys Gly Phe Asp
    130                 135                 140

Val Thr Val Lys Tyr Thr Gln Gly Ser Trp Thr Gly Phe Val Gly Glu
145                 150                 155                 160

Asp Leu Val Thr Ile Pro Lys Gly Phe Asn Thr Ser Phe Leu Val Asn
                165                 170                 175

Ile Ala Thr Ile Phe Glu Ser Glu Asn Phe Phe Leu Pro Gly Ile Lys
            180                 185                 190

Trp Asn Gly Ile Leu Gly Leu Ala Tyr Ala Thr Leu Ala Lys Pro Ser
        195                 200                 205

Ser Ser Leu Glu Thr Phe Phe Asp Ser Leu Val Thr Gln Ala Asn Ile
    210                 215                 220

Pro Asn Val Phe Ser Met Gln Met Cys Gly Ala Gly Leu Pro Val Ala
225                 230                 235                 240
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Gly Ser Gly Thr Asn Gly Gly Ser Leu Val Leu Gly Gly Ile Glu Pro
                245                 250                 255

Ser Leu Tyr Lys Gly Asp Ile Trp Tyr Thr Pro Ile Lys Glu Glu Trp
            260                 265                 270

Tyr Tyr Gln Ile Glu Ile Leu Lys Leu Glu Ile Gly Gly Gln Ser Leu
        275                 280                 285

Asn Leu Asp Cys Arg Glu Tyr Asn Ala Asp Lys Ala Ile Val Asp Ser
    290                 295                 300

Gly Thr Thr Leu Leu Arg Leu Pro Gln Lys Val Phe Asp Ala Val Val
305                 310                 315                 320

Glu Ala Val Ala Arg Ala Ser Leu Ile Pro Glu Phe Ser Asp Gly Phe
                325                 330                 335

Trp Thr Gly Ser Gln Leu Ala Cys Trp Thr Asn Ser Glu Thr Pro Trp
            340                 345                 350

Ser Tyr Phe Pro Lys Ile Ser Ile Tyr Leu Arg Asp Glu Asn Ser Ser
        355                 360                 365

Arg Ser Phe Arg Ile Thr Ile Leu Pro Gln Leu Tyr Ile Gln Pro Met
    370                 375                 380

Met Gly Ala Gly Leu Asn Tyr Glu Cys Tyr Arg Phe Gly Ile Ser Pro
385                 390                 395                 400

Ser Thr Asn Ala Leu Val Ile Gly Ala Thr Val Met Glu Gly Phe Tyr
                405                 410                 415

Val Ile Phe Asp Arg Ala Gln Lys Arg Val Gly Phe Ala Ala Ser Pro
            420                 425                 430

Cys Ala Glu Ile Ala Gly Ala Ala Val Ser Glu Ile Ser Gly Pro Phe
        435                 440                 445

Ser Thr Glu Asp Val Ala Ser Asn Cys Val Pro Ala Gln Ser Leu Ser
    450                 455                 460

Glu Pro Ile Leu Trp Ile Val Ser Tyr Ala Leu Met Ser Val Cys Gly
465                 470                 475                 480

Ala Ile Leu Leu Val Leu Ile Val Leu Leu Leu Leu Pro Phe Arg Cys
                485                 490                 495

Gln Arg Arg Pro Arg Asp Pro Glu Val Val Asn Asp Glu Ser Ser Leu
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Val Arg His Arg Trp Lys
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<210> 3
<211> 2070
<212> DNA
<213> Homo sapiens

<400> 3
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<210> 4
<211> 501
<212> PRT
<213> Homo sapiens

<400> 4
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Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser
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Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
35 40 45

Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
50 55 60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
65 70 75 80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
85 90 95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
100 105 110

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Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
        115                 120                 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
    130                 135                 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145                 150                 155                 160

Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp
                165                 170                 175

Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp
            180                 185                 190

Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro
        195                 200                 205

Asn Leu Phe Ser Leu His Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln
    210                 215                 220

Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile
225                 230                 235                 240

Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg
                245                 250                 255

Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln
            260                 265                 270

Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val
        275                 280                 285

Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala
    290                 295                 300

Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp
305                 310                 315                 320

Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr
                325                 330                 335

Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val
            340                 345                 350

Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg
        355                 360                 365

Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala
    370                 375                 380

Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile Met Glu
385                 390                 395                 400

Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala
                405                 410                 415

Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu
            420                 425                 430

Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro
        435                 440                 445
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Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val Met Ala Ala
    450                 455                 460

Ile Cys Ala Leu Phe Met Leu Pro Leu Cys Leu Met Val Cys Gln Trp
465                 470                 475                 480

Arg Cys Leu Arg Cys Leu Arg Gln Gln His Asp Asp Phe Ala Asp Asp
                485                 490                 495

Ile Ser Leu Leu Lys
            500
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<210> 5
<211> 1977
<212> DNA
<213> Homo sapiens

<400> 5

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<210> 6
<211> 476
<212> PRT
<213> Homo sapiens

<400> 6

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Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser
            20                  25                  30

Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
        35                  40                  45

Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
    50                  55                  60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
65                  70                  75                  80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
                85                  90                  95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
            100                 105                 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
        115                 120                 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
    130                 135                 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145                 150                 155                 160

Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp
                165                 170                 175

Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Leu Cys Gly
            180                 185                 190

Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly
        195                 200                 205

Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu
    210                 215                 220

Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val
225                 230                 235                 240

Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr
                245                 250                 255

Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu
            260                 265                 270

Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser
        275                 280                 285

Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val
    290                 295                 300

Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser
305                 310                 315                 320

Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile
                325                 330                 335

Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln
            340                 345                 350
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Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val
        355                 360                 365

Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala
    370                 375                 380

Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His Val His Asp Glu
385                 390                 395                 400

Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu
                405                 410                 415

Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser Thr Leu Met Thr
            420                 425                 430

Ile Ala Tyr Val Met Ala Ala Ile Cys Ala Leu Phe Met Leu Pro Leu
        435                 440                 445

Cys Leu Met Val Cys Gln Trp Arg Cys Leu Arg Cys Leu Arg Gln Gln
    450                 455                 460

His Asp Asp Phe Ala Asp Asp Ile Ser Leu Leu Lys
465                 470                 475


<210> 7
<211> 2043
<212> DNA
<213> Mus musculus

<400> 7
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gaa                                                               2043

<210> 8
<211> 501
<212> PRT
<213> Mus musculus

<400> 8
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Leu Pro Ala Gln Gly Thr His Leu Gly Ile Arg Leu Pro Leu Arg Ser
            20                  25                  30

Gly Leu Ala Gly Pro Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
        35                  40                  45

Glu Glu Ser Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
    50                  55                  60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
65                  70                  75                  80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
                85                  90                  95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
            100                 105                 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
        115                 120                 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
    130                 135                 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145                 150                 155                 160

Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp
                165                 170                 175

Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp
            180                 185                 190

Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Ile Pro
        195                 200                 205

Asn Ile Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln
    210                 215                 220

Thr Glu Ala Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile
225                 230                 235                 240

Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg
                245                 250                 255

Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln
            260                 265                 270
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Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val
        275                 280                 285

Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala
    290                 295                 300

Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp
305                 310                 315                 320

Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr
                325                 330                 335

Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val
            340                 345                 350

Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg
        355                 360                 365

Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala
    370                 375                 380

Val Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile Met Glu
385                 390                 395                 400

Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala
                405                 410                 415

Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu
            420                 425                 430

Gly Pro Phe Val Thr Ala Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro
        435                 440                 445

Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val Met Ala Ala
    450                 455                 460

Ile Cys Ala Leu Phe Met Leu Pro Leu Cys Leu Met Val Cys Gln Trp
465                 470                 475                 480

Arg Cys Leu Arg Cys Leu Arg His Gln His Asp Asp Phe Ala Asp Asp
                485                 490                 495

Ile Ser Leu Leu Lys
            500


<210> 9
<211> 2088
<212> DNA
<213> Homo sapiens

<400> 9
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<210> 10
<211> 695
<212> PRT
<213> Homo sapiens

<400> 10
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
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Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130 135 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
165 170 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
180 185 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
195 200 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210 215 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290 295 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
420 425 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
435 440 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
450 455 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465 470 475 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
485 490 495

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Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
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Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625                 630                 635                 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
                645                 650                 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
            660                 665                 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
        675                 680                 685

Phe Phe Glu Gln Met Gln Asn
    690                 695
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<210> 11
<211> 2088
<212> DNA
<213> Homo sapiens

<400> 11

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<210> 12
<211> 695
<212> PRT
<213> Homo sapiens

<400> 12

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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190
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Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
    290                 295                 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305                 310                 315                 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
                325                 330                 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
            340                 345                 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
        355                 360                 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
    370                 375                 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385                 390                 395                 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
                405                 410                 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
        435                 440                 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450                 455                 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465                 470                 475                 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
                485                 490                 495

Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
            500                 505                 510
```

```
Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590

Glu Val Asn Leu Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625                 630                 635                 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
                645                 650                 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
            660                 665                 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
        675                 680                 685

Phe Phe Glu Gln Met Gln Asn
    690                 695
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<210> 13
<211> 2088
<212> DNA
<213> Homo sapiens

<400> 13

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gtggaggttg acgccgctgt caccccagag gagcgccacc tgtccaagat gcagcagaac 2040
ggctacgaaa atccaaccta caagttcttt gagcagatgc agaactag              2088
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<210> 14
<211> 695
<212> PRT
<213> Homo sapiens

<400> 14

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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205
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```
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
    290                 295                 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305                 310                 315                 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
                325                 330                 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
            340                 345                 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
        355                 360                 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
    370                 375                 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385                 390                 395                 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
                405                 410                 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
        435                 440                 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450                 455                 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465                 470                 475                 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
                485                 490                 495

Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
            500                 505                 510

Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540
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Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625                 630                 635                 640

Ile Phe Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
                645                 650                 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
            660                 665                 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
        675                 680                 685


Phe Phe Glu Gln Met Gln Asn
    690                 695
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<210> 15
<211> 2094
<212> DNA
<213> Homo sapiens

<400> 15

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gaggagattc aggatgaagt tgatgagctg cttcagaaag agcaaaacta ttcagatgac 1500
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atcgtcatca ccttggtgat gctgaagaag aaacagtaca catccattca tcatggtgtg 1980
gtggaggttg acgccgctgt caccccagag gagcgccacc tgtccaagat gcagcagaac 2040
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<210> 16
<211> 697
<212> PRT
<213> Homo sapiens

<400> 16
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130 135 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
165 170 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
180 185 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
195 200 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210 215 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

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Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
    290                 295                 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305                 310                 315                 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
                325                 330                 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
            340                 345                 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
        355                 360                 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
    370                 375                 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385                 390                 395                 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
                405                 410                 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
        435                 440                 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450                 455                 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465                 470                 475                 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
                485                 490                 495

Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
            500                 505                 510

Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575
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Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
580 585 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
595 600 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
610 615 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
675 680 685

Phe Phe Glu Gln Met Gln Asn Lys Lys
690 695

<210> 17
<211> 2094
<212> DNA
<213> Homo sapiens

<400> 17
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ctgaacatgc acatgaatgt ccagaatggg aagtgggatt cagatccatc agggaccaaa 180
acctgcattg ataccaagga aggcatcctg cagtattgcc aagaagtcta ccctgaactg 240
cagatcacca atgtggtaga agccaaccaa ccagtgacca tccagaactg gtgcaagcgg 300
ggccgcaagc agtgcaagac ccatccccac tttgtgattc cctaccgctg cttagttggt 360
gagtttgtaa gtgatgccct tctcgttcct gacaagtgca aattcttaca ccaggagagg 420
atggatgttt gcgaaactca tcttcactgg cacaccgtcg ccaaagagac atgcagtgag 480
aagagtacca acttgcatga ctacggcatg ttgctgccct gcggaattga caagttccga 540
ggggtagagt ttgtgtgttg cccactggct gaagaaagtg acaatgtgga ttctgctgat 600
gcggaggagg atgactcgga tgtctggtgg ggcggagcag acacagacta tgcagatggg 660
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gaagccgatg atgacgagga cgatgaggat ggtgatgagg tagaggaaga ggctgaggaa 780
ccctacgaag aagccacaga gagaaccacc agcattgcca ccaccaccac caccaccaca 840
gagtctgtgg aagaggtggt tcgagttcct acaacagcag ccagtacccc tgatgccgtt 900
gacaagtatc tcgagacacc tggggatgag aatgaacatg cccatttcca gaaagccaaa 960
gagaggcttg aggccaagca ccgagagaga atgtcccagg tcatgagaga atgggaagag 1020
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gaagttgagc ctgttgatgc ccgccctgct gccgaccgag gactgaccac tcgaccaggt 1740
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cgacatgact caggatatga agttcatcat caaaaattgg tgttctttgc agaagatgtg 1860

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gtggaggttg acgccgctgt caccccagag gagcgccacc tgtccaagat gcagcagaac 2040
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<210> 18
<211> 697
<212> PRT
<213> Homo sapiens

<400> 18
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
1               5                   10                  15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205


Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270
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Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290 295 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
420 425 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
435 440 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
450 455 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465 470 475 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
485 490 495

Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
500 505 510

Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
515 520 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
530 535 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545 550 555 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
565 570 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
580 585 590

Glu Val Asn Leu Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
595 600 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
610 615 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
675 680 685

Phe Phe Glu Gln Met Gln Asn Lys Lys
690 695

<210> 19
<211> 2094
<212> DNA
<213> Homo sapiens

<400> 19
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ctgaacatgc acatgaatgt ccagaatggg aagtgggatt cagatccatc agggaccaaa 180
acctgcattg ataccaagga aggcatcctg cagtattgcc aagaagtcta ccctgaactg 240
cagatcacca atgtggtaga agccaaccaa ccagtgacca tccagaactg gtgcaagcgg 300
ggccgcaagc agtgcaagac ccatccccac tttgtgattc cctaccgctg cttagttggt 360
gagtttgtaa gtgatgccct tctcgttcct gacaagtgca aattcttaca ccaggagagg 420
atggatgttt gcgaaactca tcttcactgg cacaccgtcg ccaaagagac atgcagtgag 480
aagagtacca acttgcatga ctacggcatg ttgctgccct gcggaattga caagttccga 540
ggggtagagt ttgtgtgttg cccactggct gaagaaagtg acaatgtgga ttctgctgat 600
gcggaggagg atgactcgga tgtctggtgg ggcggagcag acacagacta tgcagatggg 660
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gaagccgatg atgacgagga cgatgaggat ggtgatgagg tagaggaaga ggctgaggaa 780
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gtggaggttg acgccgctgt caccccagag gagcgccacc tgtccaagat gcagcagaac 2040
ggctacgaaa atccaaccta caagttcttt gagcagatgc agaacaagaa gtag 2094

<210> 20
<211> 697

<212> PRT
<213> Homo sapiens

<400> 20
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110


Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
    290                 295                 300
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Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305                 310                 315                 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
                325                 330                 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
            340                 345                 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
        355                 360                 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
    370                 375                 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385                 390                 395                 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
                405                 410                 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
        435                 440                 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450                 455                 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465                 470                 475                 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
                485                 490                 495

Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
            500                 505                 510

Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625                 630                 635                 640
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Ile Phe Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
                645                 650                 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
            660                 665                 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
        675                 680                 685

Phe Phe Glu Gln Met Gln Asn Lys Lys
    690                 695

<210> 21
<211> 1341
<212> DNA
<213> Homo sapiens

<400> 21
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gacgaagagc ccgaggagcc cggccggagg ggcagctttg tggagatggt ggacaacctg 180
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ttcctgcatc gctactacca gaggcagctg tccagcacat accgggacct ccggaagggt 360
gtgtatgtgc cctacaccca gggcaagtgg gaaggggagc tgggcaccga cctggtaagc 420
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tcatccacgg gcactgttat gggagctgtt atcatggagg gcttctacgt tgtctttgat 1200
cgggcccgaa aacgaattgg ctttgctgtc agcgcttgcc atgtgcacga tgagttcagg 1260
acggcagcgg tggaaggccc ttttgtcacc ttggacatgg aagactgtgg ctacaacatt 1320
ccacagacag atgagtcatg a                                           1341

<210> 22
<211> 446
<212> PRT
<213> Homo sapiens

<400> 22
Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Gly Ser Thr Gln
  1               5                  10                  15

His Gly Ile Arg Leu Pro Leu Arg Ser Gly Leu Gly Gly Ala Pro Leu
             20                  25                  30

Gly Leu Arg Leu Pro Arg Glu Thr Asp Glu Glu Pro Glu Glu Pro Gly
         35                  40                  45

Arg Arg Gly Ser Phe Val Glu Met Val Asp Asn Leu Arg Gly Lys Ser
     50                  55                  60
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Gly Gln Gly Tyr Tyr Val Glu Met Thr Val Gly Ser Pro Pro Gln Thr
65                  70                  75                  80

Leu Asn Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val Gly Ala
                85                  90                  95

Ala Pro His Pro Phe Leu His Arg Tyr Tyr Gln Arg Gln Leu Ser Ser
            100                 105                 110

Thr Tyr Arg Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr Gln Gly
        115                 120                 125

Lys Trp Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro His Gly
    130                 135                 140

Pro Asn Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu Ser Asp
145                 150                 155                 160

Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala
                165                 170                 175

Tyr Ala Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp
            180                 185                 190

Ser Leu Val Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu His Leu
        195                 200                 205

Cys Gly Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val
    210                 215                 220

Gly Gly Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly
225                 230                 235                 240

Ser Leu Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile
                245                 250                 255

Ile Val Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys
            260                 265                 270

Glu Tyr Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu
        275                 280                 285

Arg Leu Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala
    290                 295                 300

Ala Ser Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln
305                 310                 315                 320

Leu Val Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val
                325                 330                 335

Ile Ser Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile
            340                 345                 350

Thr Ile Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val Ala Thr
        355                 360                 365

Ser Gln Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly
    370                 375                 380

Thr Val Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp
385                 390                 395                 400
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Arg Ala Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His Val His
405 410 415

Asp Glu Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp
420 425 430

Met Glu Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser
435 440 445

<210> 23
<211> 1380
<212> DNA
<213> Homo sapiens

<400> 23
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ccgcgtgaac aggacggatc cacccagcac ggcatccggc tgcccctgcg cagcggcctg 120
gggggcgccc ccctggggct gcggctgccc cgggagaccg acgaagagcc cgaggagccc 180
ggccggaggg gcagctttgt ggagatggtg gacaacctga ggggcaagtc ggggcagggc 240
tactacgtgg agatgaccgt gggcagcccc ccgcagacgc tcaacatcct ggtggataca 300
ggcagcagta actttgcagt gggtgctgcc ccccacccct tcctgcatcg ctactaccag 360
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aactgggaag gcatcctggg gctggcctat gctgagattg ccaggcctga cgactccctg 600
gagcctttct ttgactctct ggtaaagcag acccacgttc ccaacctctt ctccctgcac 660
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<210> 24
<211> 459
<212> PRT
<213> Homo sapiens

<400> 24
Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Gly Ser Met Thr
1 5 10 15

Ile Ser Asp Ser Pro Arg Glu Gln Asp Gly Ser Thr Gln His Gly Ile
20 25 30

Arg Leu Pro Leu Arg Ser Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg
35 40 45

Leu Pro Arg Glu Thr Asp Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly
50 55 60

Ser Phe Val Glu Met Val Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly
65 70 75 80

Tyr Tyr Val Glu Met Thr Val Gly Ser Pro Pro Gln Thr Leu Asn Ile
85 90 95

Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val Gly Ala Ala Pro His
100 105 110

Pro Phe Leu His Arg Tyr Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg
115 120 125

Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu
130 135 140

Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro His Gly Pro Asn Val
145 150 155 160

Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe
165 170 175

Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu
180 185 190

Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu Val
195 200 205

Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu His Leu Cys Gly Ala
210 215 220

Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly Ser
225 230 235 240

Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu Trp
245 250 255

Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg
260 265 270

Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn
275 280 285

Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro
290 295 300

Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser Ser
305 310 315 320

Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val Cys
325 330 335

Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser Leu
340 345 350

Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu
355 360 365

Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln Asp
370 375 380

Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val Met
385 390 395 400

Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala Arg
405 410 415

Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His Val His Asp Glu Phe
420 425 430

Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu Asp
        435   440   445

Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser
    450   455

<210> 25
<211> 1302
<212> DNA
<213> Homo sapiens

<400> 25
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gtggagatgg tggacaacct gaggggcaag tcggggcagg gctactacgt ggagatgacc 180
gtgggcagcc ccccgcagac gctcaacatc ctggtggata caggcagcag taactttgca 240
gtgggtgctg ccccccaccc cttcctgcat cgctactacc agaggcagct gtccagcaca 300
taccgggacc tccggaaggg tgtgtatgtg ccctacaccc agggcaagtg ggaaggggag 360
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gggctggcct atgctgagat tgccaggcct gacgactccc tggagccttt ctttgactct 540
ctggtaaagc agacccacgt tcccaacctc ttctccctgc acctttgtgg tgctggcttc 600
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gaccactcgc tgtacacagg cagtctctgg tatacaccca tccggcggga gtggtattat 720
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tacaagtttg ccatctcaca gtcatccacg ggcactgtta tgggagctgt tatcatggag 1140
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catgtgcacg atgagttcag gacggcagcg gtggaaggcc cttttgtcac cttggacatg 1260
gaagactgtg gctacaacat tccacagaca gatgagtcat ga 1302

<210> 26
<211> 433
<212> PRT
<213> Homo sapiens

<400> 26
Met Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser Gly Leu Gly Gly
1   5   10   15

Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp Glu Glu Pro Glu
            20   25   30

Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val Asp Asn Leu Arg
        35   40   45

Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr Val Gly Ser Pro
    50   55   60

Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala
65   70   75   80

Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr Tyr Gln Arg Gln
                85   90   95

Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr
            100   105   110

Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile
115 120 125

Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr
130 135 140

Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu
145 150 155 160

Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro
165 170 175

Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro Asn Leu Phe Ser
180 185 190

Leu His Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu
195 200 205

Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu
210 215 220

Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr
225 230 235 240

Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met
245 250 255

Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr
260 265 270

Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser
275 280 285

Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu
290 295 300

Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile
305 310 315 320

Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser
325 330 335

Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp
340 345 350

Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser
355 360 365

Ser Thr Gly Thr Val Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val
370 375 380

Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys
385 390 395 400

His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val
405 410 415

Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu
420 425 430

Ser

<210> 27
<211> 1278
<212> DNA
<213> Homo sapiens

<400> 27
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ccgctggact ctggtatcga aaccgacgga tcctttgtgg agatggtgga caacctgagg 120
ggcaagtcgg ggcagggcta ctacgtggag atgaccgtgg gcagccccc gcagacgctc 180
aacatcctgg tggatacagg cagcagtaac tttgcagtgg gtgctgcccc ccacccctc 240
ctgcatcgct actaccagag gcagctgtcc agcacatacc gggacctccg gaagggtgtg 300
tatgtgccct acacccaggg caagtgggaa ggggagctgg gcaccgacct ggtaagcatc 360
ccccatggcc ccaacgtcac tgtgcgtgcc aacattgctg ccatcactga atcagacaag 420
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aggcctgacg actccctgga gcctttcttt gactctctgg taaagcagac ccacgttccc 540
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ctctggtata cacccatccg gcgggagtgg tattatgagg tcatcattgt gcgggtggag 720
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gacagtggca ccaccaacct tcgtttgccc aagaaagtgt ttgaagctgc agtcaaatcc 840
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cagacagatg agtcatga 1278

<210> 28
<211> 425
<212> PRT
<213> Homo sapiens

<400> 28
Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Gly Ser Met Thr
1 5 10 15

Ile Ser Asp Ser Pro Leu Asp Ser Gly Ile Glu Thr Asp Gly Ser Phe
20 25 30

Val Glu Met Val Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr
35 40 45

Val Glu Met Thr Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val
50 55 60

Asp Thr Gly Ser Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe
65 70 75 80

Leu His Arg Tyr Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu
85 90 95

Arg Lys Gly Val Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu
100 105 110

Leu Gly Thr Asp Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val
115 120 125

Arg Ala Asn Ile Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn
130 135 140

Gly Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala
145 150 155 160

Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln
165 170 175

Thr His Val Pro Asn Leu Phe Ser Leu His Leu Cys Gly Ala Gly Phe
180 185 190

Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile
195 200 205

Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr
210 215 220

Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu
225 230 235 240

Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp
245 250 255

Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys
260 265 270

Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu
275 280 285

Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln
290 295 300

Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu
305 310 315 320

Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln
325 330 335

Gln Tyr Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys
340 345 350

Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala
355 360 365

Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg
370 375 380

Ile Gly Phe Ala Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr
385 390 395 400

Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly
405 410 415

Tyr Asn Ile Pro Gln Thr Asp Glu Ser
420 425

<210> 29
<211> 1362
<212> DNA
<213> Homo sapiens

<400> 29
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ggcacccagc acggcatccg gctgcccctg cgcagcggcc tgggggggcgc ccccctgggg 120

ctgcggctgc cccgggagac cgacgaagag cccgaggagc ccggccggag gggcagcttt 180
gtggagatgg tggacaacct gaggggcaag tcggggcagg gctactacgt ggagatgacc 240
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gaagactgtg gctacaacat tccacagaca gatgagtcat ga 1362

<210> 30
<211> 453
<212> PRT
<213> Homo sapiens

<400> 30
Met Ala Gln Ala Leu Pro Trp Leu Leu Leu Trp Met Gly Ala Gly Val
1 5 10 15

Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser
20 25 30

Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
35 40 45

Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
50 55 60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
65 70 75 80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
85 90 95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
100 105 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
115 120 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
130 135 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145 150 155 160

Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp
165 170 175

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Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp
            180                 185                 190

Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro
        195                 200                 205

Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln
    210                 215                 220

Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile
225                 230                 235                 240

Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg
                245                 250                 255

Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln
            260                 265                 270

Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val
        275                 280                 285

Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala
    290                 295                 300

Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp
305                 310                 315                 320

Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr
                325                 330                 335

Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val
            340                 345                 350

Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg
        355                 360                 365

Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala
    370                 375                 380

Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile Met Glu
385                 390                 395                 400

Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala
                405                 410                 415

Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu
            420                 425                 430

Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro
        435                 440                 445

Gln Thr Asp Glu Ser
    450
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<210> 31
<211> 1380
<212> DNA
<213> Homo sapiens

<400> 31
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ctgcggctgc cccgggagac cgacgaagag cccgaggagc ccggccggag gggcagcttt 180
gtggagatgg tggacaacct gaggggcaag tcggggcagg gctactacgt ggagatgacc 240
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gaccactcgc tgtacacagg cagtctctgg tatacaccca tccggcggga gtggtattat 780
gaggtcatca ttgtgcgggt ggagatcaat ggacaggatc tgaaaatgga ctgcaaggag 840
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atccttccgc agcaatacct gcggccagtg gaagatgtgg ccacgtccca agacgactgt 1140
tacaagtttg ccatctcaca gtcatccacg ggcactgtta tgggagctgt tatcatggag 1200
ggcttctacg ttgtctttga tcgggcccga aaacgaattg gctttgctgt cagcgcttgc 1260
catgtgcacg atgagttcag gacggcagcg gtggaaggcc cttttgtcac cttggacatg 1320
gaagactgtg gctacaacat tccacagaca gatgagtcac agcagcagca gcagcagtga 1380
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<210> 32
<211> 459
<212> PRT
<213> Homo sapiens

<400> 32

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Met Ala Gln Ala Leu Pro Trp Leu Leu Leu Trp Met Gly Ala Gly Val
1               5                   10                  15

Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser
            20                  25                  30

Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
        35                  40                  45

Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
    50                  55                  60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
65                  70                  75                  80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
                85                  90                  95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
            100                 105                 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
        115                 120                 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
    130                 135                 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145                 150                 155                 160

Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp
                165                 170                 175
```

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Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp
            180                 185                 190

Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro
        195                 200                 205

Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln
    210                 215                 220

Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile
225                 230                 235                 240

Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg
                245                 250                 255

Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln
            260                 265                 270

Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val
        275                 280                 285

Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala
    290                 295                 300

Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp
305                 310                 315                 320

Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr
                325                 330                 335

Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val
            340                 345                 350

Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg
        355                 360                 365

Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala
    370                 375                 380

Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile Met Glu
385                 390                 395                 400

Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala
                405                 410                 415

Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu
            420                 425                 430

Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro
        435                 440                 445

Gln Thr Asp Glu Ser His His His His His His
    450                 455
```

<210> 33
<211> 25
<212> PRT
<213> Homo sapiens

<400> 33

Ser Glu Gln Gln Arg Arg Pro Arg Asp Pro Glu Val Val Asn Asp Glu
1 5 10 15

Ser Ser Leu Val Arg His Arg Trp Lys
20 25

<210> 34
<211> 19
<212> PRT
<213> Homo sapiens

<400> 34
Ser Glu Gln Leu Arg Gln Gln His Asp Asp Phe Ala Asp Asp Ile Ser
1 5 10 15

Leu Leu Lys

<210> 35
<211> 29
<212> DNA
<213> Homo sapiens

<400> 35
gtggatccac ccagcacggc atccggctg 29

<210> 36
<211> 36
<212> DNA
<213> Homo sapiens

<400> 36
gaaagctttc atgactcatc tgtctgtgga atgttg 36

<210> 37
<211> 39
<212> DNA
<213> Homo sapiens

<400> 37
gatcgatgac tatctctgac tctccgcgtg aacaggacg 39

<210> 38
<211> 39
<212> DNA
<213> Homo sapiens

<400> 38
gatccgtcct gttcacgcgg agagtcagag atagtcatc 39

<210> 39
<211> 77
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Hu-Asp2

<400> 39
cggcatccgg ctgcccctgc gtagcggtct gggtggtgct ccactgggtc tgcgtctgcc 60
ccgggagacc gacgaag 77

```
<210> 40
<211> 77
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Hu-Asp2

<400> 40
cttcgtcggt ctcccggggc agacgcagac ccagtggagc accacccaga ccgctacgca 60
ggggcagccg gatgccg                                                77

<210> 41
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Caspase 8
      Cleavage Site

<400> 41
gatcgatgac tatctctgac tctccgctgg actctggtat cgaaaccgac g          51

<210> 42
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Caspase 8
      Cleavage Site

<400> 42
gatccgtcgg tttcgatacc agagtccagc ggagagtcag agatagtcat c          51

<210> 43
<211> 32
<212> DNA
<213> Homo sapiens

<400> 43
aaggatcctt tgtggagatg gtggacaacc tg                               32

<210> 44
<211> 36
<212> DNA
<213> Homo sapiens

<400> 44
gaaagctttc atgactcatc tgtctgtgga atgttg                           36

<210> 45
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 6-His tag

<400> 45
gatcgcatca tcaccatcac catg                                        24
```

<210> 46
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 6-His tag

<400> 46
gatccatggt gatggtgatg atgc 24

<210> 47
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 47
gactgaccac tcgaccaggt tc 22

<210> 48
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 48
cgaattaaat tccagcacac tggctacttc ttgttctgca tctcaaagaa c 51

<210> 49
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 49
cgaattaaat tccagcacac tggcta 26

<210> 50
<211> 1287
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Hu-Asp2(b)
delta TM

<400> 50
atggcccaag ccctgccctg gctcctgctg tggatgggcg cgggagtgct gcctgcccac 60
ggcacccagc acggcatccg gctgcccctg cgcagcggcc tgggggggcgc ccccctgggg 120
ctgcggctgc cccgggagac cgacgaagag cccgaggagc ccggccggag gggcagcttt 180
gtggagatgg tggacaacct gaggggcaag tcggggcagg gctactacgt ggagatgacc 240
gtgggcagcc ccccgcagac gctcaacatc ctggtggata caggcagcag taactttgca 300
gtgggtgctg ccccccaccc cttcctgcat cgctactacc agaggcagct gtccagcaca 360
taccgggacc tccggaaggg tgtgtatgtg ccctacaccc agggcaagtg ggaaggggag 420
ctgggcaccg acctggtaag catcccccat ggccccaacg tcactgtgcg tgccaacatt 480
gctgccatca ctgaatcaga caagttcttc atcaacggct ccaactggga aggcatcctg 540

```
gggctggcct atgctgagat tgccaggctt tgtggtgctg gcttcccccct caaccagtct 600
gaagtgctgg cctctgtcgg agggagcatg atcattggag gtatcgacca ctcgctgtac 660
acaggcagtc tctggtatac acccatccgg cgggagtggt attatgaggt catcattgtg 720
cgggtggaga tcaatggaca ggatctgaaa atggactgca aggagtacaa ctatgacaag 780
agcattgtgg acagtggcac caccaacctt cgtttgccca agaaagtgtt tgaagctgca 840
gtcaaatcca tcaaggcagc ctcctccacg gagaagttcc ctgatggttt ctggctagga 900
gagcagctgg tgtgctggca agcaggcacc acccccttgga acatttcccc agtcatctca 960
ctctacctaa tgggtgaggt taccaaccag tccttccgca tcaccatcct tccgcagcaa 1020
tacctgcggc cagtggaaga tgtggccacg tcccaagacg actgttacaa gtttgccatc 1080
tcacagtcat ccacgggcac tgttatggga gctgttatca tggagggctt ctacgttgtc 1140
tttgatcggg cccgaaaacg aattggcttt gctgtcagcg cttgccatgt gcacgatgag 1200
ttcaggacgg cagcggtgga aggccctttt gtcaccttgg acatggaaga ctgtggctac 1260
aacattccac agacagatga gtcatga                                    1287

<210> 51
<211> 428
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Hu-Asp2(b)
      delta TM

<400> 51
Met Ala Gln Ala Leu Pro Trp Leu Leu Leu Trp Met Gly Ala Gly Val
  1               5                  10                  15

Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser
             20                  25                  30

Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
         35                  40                  45

Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
     50                  55                  60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
 65                  70                  75                  80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
                 85                  90                  95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
            100                 105                 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
        115                 120                 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
    130                 135                 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145                 150                 155                 160

Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp
                165                 170                 175

Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Leu Cys Gly
            180                 185                 190

Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly
        195                 200                 205
```

Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu
210 215 220

Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val
225 230 235 240

Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr
245 250 255

Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu
260 265 270

Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser
275 280 285

Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val
290 295 300

Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser
305 310 315 320

Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile
325 330 335

Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln
340 345 350

Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val
355 360 365

Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala
370 375 380

Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His Val His Asp Glu
385 390 395 400

Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu
405 410 415

Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser
420 425

<210> 52
<211> 1305
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Hu-Asp2(b) delta TM

<400> 52
atggcccaag ccctgccctg gctcctgctg tggatgggcg cgggagtgct gcctgcccac 60
ggcacccagc acggcatccg gctgcccctg cgcagcggcc tgggggcgc cccctgggg 120
ctgcggctgc cccgggagac cgacgaagag cccgaggagc ccggccggag gggcagcttt 180
gtggagatgg tggacaacct gaggggcaag tcggggcagg gctactacgt ggagatgacc 240
gtgggcagcc ccccgcagac gctcaacatc ctggtggata caggcagcag taactttgca 300
gtgggtgctg ccccccaccc cttcctgcat cgctactacc agaggcagct gtccagcaca 360
taccgggacc tccggaaggg tgtgtatgtg ccctacaccc agggcaagtg ggaaggggag 420
ctgggcaccg acctggtaag catcccccat ggccccaacg tcactgtgcg tgccaacatt 480
gctgccatca ctgaatcaga caagttcttc atcaacggct ccaactggga aggcatcctg 540
gggctggcct atgctgagat tgccaggctt tgtggtgctg gcttcccct caaccagtct 600

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gaagtgctgg cctctgtcgg agggagcatg atcattggag gtatcgacca ctcgctgtac 660
acaggcagtc tctggtatac acccatccgg cgggagtggt attatgaggt catcattgtg 720
cgggtggaga tcaatggaca ggatctgaaa atggactgca aggagtacaa ctatgacaag 780
agcattgtgg acagtggcac caccaacctt cgtttgccca agaaagtgtt tgaagctgca 840
gtcaaatcca tcaaggcagc ctcctccacg gagaagttcc ctgatggttt ctggctagga 900
gagcagctgg tgtgctggca agcaggcacc accccttgga acattttccc agtcatctca 960
ctctacctaa tgggtgaggt taccaaccag tccttccgca tcaccatcct tccgcagcaa 1020
tacctgcggc cagtggaaga tgtggccacg tcccaagacg actgttacaa gtttgccatc 1080
tcacagtcat ccacgggcac tgttatggga gctgttatca tggagggctt ctacgttgtc 1140
tttgatcggg cccgaaaacg aattggcttt gctgtcagcg cttgccatgt gcacgatgag 1200
ttcaggacgg cagcggtgga aggccctttt gtcaccttgg acatggaaga ctgtggctac 1260
aacattccac agacagatga gtcacagcag cagcagcagc agtga                1305
```

<210> 53
<211> 434
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Hu-Asp2(b)
delta TM

<400> 53

```
Met Ala Gln Ala Leu Pro Trp Leu Leu Leu Trp Met Gly Ala Gly Val
  1               5                  10                  15

Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser
             20                  25                  30

Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
         35                  40                  45

Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
     50                  55                  60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
 65                  70                  75                  80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
                 85                  90                  95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
            100                 105                 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
        115                 120                 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
    130                 135                 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145                 150                 155                 160

Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp
                165                 170                 175

Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Leu Cys Gly
            180                 185                 190

Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly
        195                 200                 205
```

```
Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu
    210                 215                 220

Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val
225                 230                 235                 240

Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr
                245                 250                 255

Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu
            260                 265                 270

Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser
        275                 280                 285

Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val
    290                 295                 300

Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser
305                 310                 315                 320

Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile
                325                 330                 335

Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln
            340                 345                 350

Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val
        355                 360                 365

Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala
    370                 375                 380

Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His Val His Asp Glu
385                 390                 395                 400

Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu
                405                 410                 415

Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser His His His His
            420                 425                 430

His His


<210> 54
<211> 2310
<212> DNA
<213> Homo sapiens

<400> 54
atgctgcccg gtttggcact gctcctgctg gccgcctgga cggctcgggc gctggaggta   60
cccactgatg gtaatgctgg cctgctggct gaaccccaga ttgccatgtt ctgtggcaga  120
ctgaacatgc acatgaatgt ccagaatggg aagtgggatt cagatccatc agggaccaaa  180
acctgcattg ataccaagga aggcatcctg cagtattgcc aagaagtcta ccctgaactg  240
cagatcacca atgtggtaga agccaaccaa ccagtgacca tccagaactg gtgcaagcgg  300
ggccgcaagc agtgcaagac ccatccccac tttgtgattc cctaccgctg cttagttggt  360
gagtttgtaa gtgatgccct tctcgttcct gacaagtgca aattcttaca ccaggagagg  420
atggatgttt gcgaaactca tcttcactgg cacaccgtcg ccaaagagac atgcagtgag  480
aagagtacca acttgcatga ctacggcatg ttgctgccct gcggaattga caagttccga  540
ggggtagagt ttgtgtgttg cccactggct gaagaaagtg acaatgtgga ttctgctgat  600
```

```
gcggaggagg atgactcgga tgtctggtgg ggcggagcag acacagacta tgcagatggg 660
agtgaagaca aagtagtaga agtagcagag gaggaagaag tggctgaggt ggaagaagaa 720
gaagccgatg atgacgagga cgatgaggat ggtgatgagg tagaggaaga ggctgaggaa 780
ccctacgaag aagccacaga gagaaccacc agcattgcca ccaccaccac caccaccaca 840
gagtctgtgg aagaggtggt tcgagaggtg tgctctgaac aagccgagac ggggccgtgc 900
cgagcaatga tctcccgctg gtactttgat gtgactgaag ggaagtgtgc cccattcttt 960
tacggcggat gtggcggcaa ccggaacaac tttgacacag aagagtactg catggccgtg 1020
tgtggcagcg ccatgtccca aagtttactc aagactaccc aggaacctct tggccgagat 1080
cctgttaaac ttcctacaac agcagccagt acccctgatg ccgttgacaa gtatctcgag 1140
acacctgggg atgagaatga acatgcccat ttccagaaag ccaaagagag gcttgaggcc 1200
aagcaccgag agagaatgtc ccaggtcatg agagaatggg aagaggcaga acgtcaagca 1260
aagaacttgc ctaaagctga taagaaggca gttatccagc atttccagga gaaagtggaa 1320
tctttggaac aggaagcagc caacgagaga cagcagctgg tggagacaca catggccaga 1380
gtggaagcca tgctcaatga ccgccgccgc ctggccctgg agaactacat caccgctctg 1440
caggctgttc ctcctcggcc tcgtcacgtg ttcaatatgc taaagaagta tgtccgcgca 1500
gaacagaagg acagacagca caccctaaag catttcgagc atgtgcgcat ggtggatccc 1560
aagaaagccg ctcagatccg gtcccaggtt atgacacacc tccgtgtgat ttatgagcgc 1620
atgaatcagt ctctctccct gctctacaac gtgcctgcag tggccgagga gattcaggat 1680
gaagttgatg agctgcttca gaaagagcaa aactattcag atgacgtctt ggccaacatg 1740
attagtgaac caaggatcag ttacggaaac gatgctctca tgccatcttt gaccgaaacg 1800
aaaaccaccg tggagctcct tcccgtgaat ggagagttca gcctggacga tctccagccg 1860
tggcattctt ttggggctga ctctgtgcca gccaacacag aaaacgaagt tgagcctgtt 1920
gatgcccgcc ctgctgccga ccgaggactg accactcgac caggttctgg gttgacaaat 1980
atcaagacgg aggagatctc tgaagtgaag atggatgcag aattccgaca tgactcagga 2040
tatgaagttc atcatcaaaa attggtgttc tttgcagaag atgtgggttc aaacaaaggt 2100
gcaatcattg gactcatggt gggcggtgtt gtcatagcga cagtgatcgt catcaccttg 2160
gtgatgctga agaagaaaca gtacacatcc attcatcatg gtgtggtgga ggttgacgcc 2220
gctgtcaccc cagaggagcg ccacctgtcc aagatgcagc agaacggcta cgaaaatcca 2280
acctacaagt tctttgagca gatgcagaac                                  2310
```

<210> 55
<211> 770
<212> PRT
<213> Homo sapiens

<400> 55

```
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
1               5                   10                  15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140
```

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
165 170 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
180 185 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
195 200 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210 215 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
290 295 300

Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305 310 315 320

Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu Tyr
325 330 335

Cys Met Ala Val Cys Gly Ser Ala Met Ser Gln Ser Leu Leu Lys Thr
340 345 350

Thr Gln Glu Pro Leu Ala Arg Asp Pro Val Lys Leu Pro Thr Thr Ala
355 360 365

Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp
370 375 380

Glu Asn Glu His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala
385 390 395 400

Lys His Arg Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala
405 410 415

Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile
420 425 430

Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn
435 440 445

Glu Arg Gln Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met
450 455 460

Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu
465 470 475 480

```
Gln Ala Val Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys
                485                 490                 495

Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe
            500                 505                 510

Glu His Val Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser
        515                 520                 525

Gln Val Met Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser
    530                 535                 540

Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp
545                 550                 555                 560

Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val
                565                 570                 575

Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala
            580                 585                 590

Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro
        595                 600                 605

Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe
    610                 615                 620

Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val
625                 630                 635                 640

Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser
                645                 650                 655

Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp
            660                 665                 670

Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu
        675                 680                 685

Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly
    690                 695                 700

Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu
705                 710                 715                 720

Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val
                725                 730                 735

Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met
            740                 745                 750

Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met
        755                 760                 765

Gln Asn
    770
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<210> 56
<211> 2253
<212> DNA
<213> Homo sapiens

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ctgaacatgc acatgaatgt ccagaatggg aagtgggatt cagatccatc agggaccaaa 180
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cagatcacca atgtggtaga agccaaccaa ccagtgacca tccagaactg gtgcaagcgg 300
ggccgcaagc agtgcaagac ccatccccac tttgtgattc cctaccgctg cttagttggt 360
gagtttgtaa gtgatgccct tctcgttcct gacaagtgca aattcttaca ccaggagagg 420
atggatgttt gcgaaactca tcttcactgg cacaccgtcg ccaaagagac atgcagtgag 480
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ggggtagagt ttgtgtgttg cccactggct gaagaaagtg acaatgtgga ttctgctgat 600
gcggaggagg atgactcgga tgtctggtgg ggcggagcag acacagacta tgcagatggg 660
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<211> 751
<212> PRT
<213> Homo sapiens

<400> 57
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

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Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                 85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
    290                 295                 300

Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305                 310                 315                 320

Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu Tyr
                325                 330                 335


Cys Met Ala Val Cys Gly Ser Ala Ile Pro Thr Thr Ala Ala Ser Thr
            340                 345                 350

Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp Glu Asn Glu
        355                 360                 365

His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala Lys His Arg
    370                 375                 380

Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala Glu Arg Gln
385                 390                 395                 400

Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile Gln His Phe
                405                 410                 415
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Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn Glu Arg Gln
420 425 430

Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met Leu Asn Asp
435 440 445

Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu Gln Ala Val
450 455 460

Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys Tyr Val Arg
465 470 475 480

Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe Glu His Val
485 490 495

Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser Gln Val Met
500 505 510

Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu
515 520 525

Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp Glu Val Asp
530 535 540

Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn
545 550 555 560

Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro
565 570 575

Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro Val Asn Gly
580 585 590

Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp
595 600 605

Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg
610 615 620

Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr
625 630 635 640

Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe
645 650 655

Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe
660 665 670

Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val
675 680 685

Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu
690 695 700

Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp
705 710 715 720

Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn
725 730 735

Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn
740 745 750

<210> 58
<211> 2316
<212> DNA
<213> Homo sapiens

<400> 58
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cagatcacca atgtggtaga agccaaccaa ccagtgacca tccagaactg gtgcaagcgg 300
ggccgcaagc agtgcaagac ccatccccac tttgtgattc cctaccgctg cttagttggt 360
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atggatgttt gcgaaactca tcttcactgg cacaccgtcg ccaaagagac atgcagtgag 480
aagagtacca acttgcatga ctacggcatg ttgctgccct gcggaattga caagttccga 540
ggggtagagt ttgtgtgttg cccactggct gaagaaagtg acaatgtgga ttctgctgat 600
gcggaggagg atgactcgga tgtctggtgg ggcggagcag acacagacta tgcagatggg 660
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<210> 59
<211> 772
<212> PRT
<213> Homo sapiens

<400> 59
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45
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Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
     50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
    290                 295                 300

Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305                 310                 315                 320

Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu Tyr
                325                 330                 335

Cys Met Ala Val Cys Gly Ser Ala Met Ser Gln Ser Leu Leu Lys Thr
            340                 345                 350

Thr Gln Glu Pro Leu Ala Arg Asp Pro Val Lys Leu Pro Thr Thr Ala
        355                 360                 365

Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp
    370                 375                 380
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Glu Asn Glu His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala
385 390 395 400

Lys His Arg Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala
405 410 415

Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile
420 425 430

Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn
435 440 445

Glu Arg Gln Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met
450 455 460

Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu
465 470 475 480

Gln Ala Val Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys
485 490 495

Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe
500 505 510

Glu His Val Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser
515 520 525

Gln Val Met Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser
530 535 540

Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp
545 550 555 560

Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val
565 570 575

Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala
580 585 590

Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro
595 600 605

Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe
610 615 620

Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val
625 630 635 640

Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser
645 650 655

Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp
660 665 670

Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu
675 680 685

Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly
690 695 700

Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu
705 710 715 720

```
Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val
                725                 730                 735

Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met
            740                 745                 750

Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met
        755                 760                 765

Gln Asn Lys Lys
    770
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<210> 60
<211> 2259
<212> DNA
<213> Homo sapiens

<400> 60

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cagatcacca atgtggtaga agccaaccaa ccagtgacca tccagaactg gtgcaagcgg 300
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aagagtacca acttgcatga ctacggcatg ttgctgccct gcggaattga caagttccga 540
ggggtagagt ttgtgtgttg cccactggct gaagaaagtg acaatgtgga ttctgctgat 600
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<210> 61
<211> 753
<212> PRT
<213> Homo sapiens

<400> 61
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130 135 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
165 170 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
180 185 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
195 200 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210 215 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
290 295 300

Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305 310 315 320

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Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu Tyr
                325                 330                 335

Cys Met Ala Val Cys Gly Ser Ala Ile Pro Thr Thr Ala Ala Ser Thr
            340                 345                 350

Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp Glu Asn Glu
        355                 360                 365

His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala Lys His Arg
    370                 375                 380

Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala Glu Arg Gln
385                 390                 395                 400

Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile Gln His Phe
                405                 410                 415

Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn Glu Arg Gln
            420                 425                 430

Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met Leu Asn Asp
        435                 440                 445

Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu Gln Ala Val
    450                 455                 460

Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys Tyr Val Arg
465                 470                 475                 480

Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe Glu His Val
                485                 490                 495

Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser Gln Val Met
            500                 505                 510

Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu
        515                 520                 525

Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp Glu Val Asp
    530                 535                 540

Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn
545                 550                 555                 560

Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro
                565                 570                 575

Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro Val Asn Gly
            580                 585                 590

Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp
        595                 600                 605

Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg
    610                 615                 620

Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr
625                 630                 635                 640

Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe
                645                 650                 655
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Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe
            660                 665                 670

Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val
        675                 680                 685

Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu
    690                 695                 700

Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp
705                 710                 715                 720

Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn
                725                 730                 735

Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn Lys
            740                 745                 750

Lys


<210> 62
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic

<400> 62
Leu Glu Val Leu Phe Gln Gly Pro
  1               5


<210> 63
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic

<400> 63
Ser Glu Val Asn Leu Asp Ala Glu Phe Arg
  1               5                  10


<210> 64
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic

<400> 64
Ser Glu Val Lys Met Asp Ala Glu Phe Arg
  1               5                  10


<210> 65
<211> 15
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic

<400> 65
Arg Arg Gly Gly Val Val Ile Ala Thr Val Ile Val Gly Glu Arg
1 5 10 15

<210> 66
<211> 518
<212> PRT
<213> Homo sapiens

<400> 66
Met Gly Ala Leu Ala Arg Ala Leu Leu Leu Pro Leu Leu Ala Gln Trp
1 5 10 15

Leu Leu Arg Ala Ala Pro Glu Leu Ala Pro Ala Pro Phe Thr Leu Pro
20 25 30

Leu Arg Val Ala Ala Ala Thr Asn Arg Val Val Ala Pro Thr Pro Gly
35 40 45

Pro Gly Thr Pro Ala Glu Arg His Ala Asp Gly Leu Ala Leu Ala Leu
50 55 60

Glu Pro Ala Leu Ala Ser Pro Ala Gly Ala Ala Asn Phe Leu Ala Met
65 70 75 80

Val Asp Asn Leu Gln Gly Asp Ser Gly Arg Gly Tyr Tyr Leu Glu Met
85 90 95

Leu Ile Gly Thr Pro Pro Gln Lys Leu Gln Ile Leu Val Asp Thr Gly
100 105 110

Ser Ser Asn Phe Ala Val Ala Gly Thr Pro His Ser Tyr Ile Asp Thr
115 120 125

Tyr Phe Asp Thr Glu Arg Ser Ser Thr Tyr Arg Ser Lys Gly Phe Asp
130 135 140

Val Thr Val Lys Tyr Thr Gln Gly Ser Trp Thr Gly Phe Val Gly Glu
145 150 155 160

Asp Leu Val Thr Ile Pro Lys Gly Phe Asn Thr Ser Phe Leu Val Asn
165 170 175

Ile Ala Thr Ile Phe Glu Ser Glu Asn Phe Phe Leu Pro Gly Ile Lys
180 185 190

Trp Asn Gly Ile Leu Gly Leu Ala Tyr Ala Thr Leu Ala Lys Pro Ser
195 200 205

Ser Ser Leu Glu Thr Phe Phe Asp Ser Leu Val Thr Gln Ala Asn Ile
210 215 220

Pro Asn Val Phe Ser Met Gln Met Cys Gly Ala Gly Leu Pro Val Ala
225 230 235 240

Gly Ser Gly Thr Asn Gly Gly Ser Leu Val Leu Gly Gly Ile Glu Pro
245 250 255

Ser Leu Tyr Lys Gly Asp Ile Trp Tyr Thr Pro Ile Lys Glu Glu Trp
260 265 270

Tyr Tyr Gln Ile Glu Ile Leu Lys Leu Glu Ile Gly Gly Gln Ser Leu
275 280 285

Asn Leu Asp Cys Arg Glu Tyr Asn Ala Asp Lys Ala Ile Val Asp Ser
290 295 300

Gly Thr Thr Leu Leu Arg Leu Pro Gln Lys Val Phe Asp Ala Val Val
305 310 315 320

Glu Ala Val Ala Arg Ala Ser Leu Ile Pro Glu Phe Ser Asp Gly Phe
325 330 335

Trp Thr Gly Ser Gln Leu Ala Cys Trp Thr Asn Ser Glu Thr Pro Trp
340 345 350

Ser Tyr Phe Pro Lys Ile Ser Ile Tyr Leu Arg Asp Glu Asn Ser Ser
355 360 365

Arg Ser Phe Arg Ile Thr Ile Leu Pro Gln Leu Tyr Ile Gln Pro Met
370 375 380

Met Gly Ala Gly Leu Asn Tyr Glu Cys Tyr Arg Phe Gly Ile Ser Pro
385 390 395 400

Ser Thr Asn Ala Leu Val Ile Gly Ala Thr Val Met Glu Gly Phe Tyr
405 410 415

Val Ile Phe Asp Arg Ala Gln Lys Arg Val Gly Phe Ala Ala Ser Pro
420 425 430

Cys Ala Glu Ile Ala Gly Ala Ala Val Ser Glu Ile Ser Gly Pro Phe
435 440 445

Ser Thr Glu Asp Val Ala Ser Asn Cys Val Pro Ala Gln Ser Leu Ser
450 455 460

Glu Pro Ile Leu Trp Ile Val Ser Tyr Ala Leu Met Ser Val Cys Gly
465 470 475 480

Ala Ile Leu Leu Val Leu Ile Val Leu Leu Leu Leu Pro Phe Arg Cys
485 490 495

Gln Arg Arg Pro Arg Asp Pro Glu Val Val Asn Asp Glu Ser Ser Leu
500 505 510

Val Arg His Arg Trp Lys
515

<210> 67
<211> 475
<212> PRT
<213> Homo sapiens

<400> 67
Met Gly Ala Leu Ala Arg Ala Leu Leu Leu Pro Leu Leu Ala Gln Trp
1 5 10 15

Leu Leu Arg Ala Ala Pro Glu Leu Ala Pro Ala Pro Phe Thr Leu Pro
20 25 30

Leu Arg Val Ala Ala Ala Thr Asn Arg Val Val Ala Pro Thr Pro Gly
35 40 45

Pro Gly Thr Pro Ala Glu Arg His Ala Asp Gly Leu Ala Leu Ala Leu
50 55 60

Glu Pro Ala Leu Ala Ser Pro Ala Gly Ala Ala Asn Phe Leu Ala Met
65 70 75 80

Val Asp Asn Leu Gln Gly Asp Ser Gly Arg Gly Tyr Tyr Leu Glu Met
85 90 95

Leu Ile Gly Thr Pro Pro Gln Lys Leu Gln Ile Leu Val Asp Thr Gly
100 105 110

Ser Ser Asn Phe Ala Val Ala Gly Thr Pro His Ser Tyr Ile Asp Thr
115 120 125

Tyr Phe Asp Thr Glu Arg Ser Ser Thr Tyr Arg Ser Lys Gly Phe Asp
130 135 140

Val Thr Val Lys Tyr Thr Gln Gly Ser Trp Thr Gly Phe Val Gly Glu
145 150 155 160

Asp Leu Val Thr Ile Pro Lys Gly Phe Asn Thr Ser Phe Leu Val Asn
165 170 175

Ile Ala Thr Ile Phe Glu Ser Glu Asn Phe Phe Leu Pro Gly Ile Lys
180 185 190

Trp Asn Gly Ile Leu Gly Leu Ala Tyr Ala Thr Leu Ala Lys Pro Ser
195 200 205

Ser Ser Leu Glu Thr Phe Phe Asp Ser Leu Val Thr Gln Ala Asn Ile
210 215 220

Pro Asn Val Phe Ser Met Gln Met Cys Gly Ala Gly Leu Pro Val Ala
225 230 235 240

Gly Ser Gly Thr Asn Gly Gly Ser Leu Val Leu Gly Gly Ile Glu Pro
245 250 255

Ser Leu Tyr Lys Gly Asp Ile Trp Tyr Thr Pro Ile Lys Glu Glu Trp
260 265 270

Tyr Tyr Gln Ile Glu Ile Leu Lys Leu Glu Ile Gly Gly Gln Ser Leu
275 280 285

Asn Leu Asp Cys Arg Glu Tyr Asn Ala Asp Lys Ala Ile Val Asp Ser
290 295 300

Gly Thr Thr Leu Leu Arg Leu Pro Gln Lys Val Phe Asp Ala Val Val
305 310 315 320

Glu Ala Val Ala Arg Ala Ser Leu Ile Pro Glu Phe Ser Asp Gly Phe
325 330 335

Trp Thr Gly Ser Gln Leu Ala Cys Trp Thr Asn Ser Glu Thr Pro Trp
340 345 350

Ser Tyr Phe Pro Lys Ile Ser Ile Tyr Leu Arg Asp Glu Asn Ser Ser
355 360 365

Arg Ser Phe Arg Ile Thr Ile Leu Pro Gln Leu Tyr Ile Gln Pro Met
370 375 380

Met Gly Ala Gly Leu Asn Tyr Glu Cys Tyr Arg Phe Gly Ile Ser Pro
385 390 395 400

Ser Thr Asn Ala Leu Val Ile Gly Ala Thr Val Met Glu Gly Phe Tyr
405 410 415

Val Ile Phe Asp Arg Ala Gln Lys Arg Val Gly Phe Ala Ala Ser Pro
420 425 430

Cys Ala Glu Ile Ala Gly Ala Ala Val Ser Glu Ile Ser Gly Pro Phe
435 440 445

Ser Thr Glu Asp Val Ala Ser Asn Cys Val Pro Ala Gln Ser Leu Ser
450 455 460

Glu Pro Ile Leu Trp His His His His His His
465 470 475

<210> 68
<211> 413
<212> PRT
<213> Homo sapiens

<400> 68
Ala Leu Glu Pro Ala Leu Ala Ser Pro Ala Gly Ala Ala Asn Phe Leu
1 5 10 15

Ala Met Val Asp Asn Leu Gln Gly Asp Ser Gly Arg Gly Tyr Tyr Leu
20 25 30

Glu Met Leu Ile Gly Thr Pro Pro Gln Lys Leu Gln Ile Leu Val Asp
35 40 45

Thr Gly Ser Ser Asn Phe Ala Val Ala Gly Thr Pro His Ser Tyr Ile
50 55 60

Asp Thr Tyr Phe Asp Thr Glu Arg Ser Ser Thr Tyr Arg Ser Lys Gly
65 70 75 80

Phe Asp Val Thr Val Lys Tyr Thr Gln Gly Ser Trp Thr Gly Phe Val
85 90 95

Gly Glu Asp Leu Val Thr Ile Pro Lys Gly Phe Asn Thr Ser Phe Leu
100 105 110

Val Asn Ile Ala Thr Ile Phe Glu Ser Glu Asn Phe Phe Leu Pro Gly
115 120 125

Ile Lys Trp Asn Gly Ile Leu Gly Leu Ala Tyr Ala Thr Leu Ala Lys
130 135 140

Pro Ser Ser Ser Leu Glu Thr Phe Phe Asp Ser Leu Val Thr Gln Ala
145 150 155 160

Asn Ile Pro Asn Val Phe Ser Met Gln Met Cys Gly Ala Gly Leu Pro
165 170 175

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Val Ala Gly Ser Gly Thr Asn Gly Gly Ser Leu Val Leu Gly Gly Ile
            180                 185                 190

Glu Pro Ser Leu Tyr Lys Gly Asp Ile Trp Tyr Thr Pro Ile Lys Glu
        195                 200                 205

Glu Trp Tyr Tyr Gln Ile Glu Ile Leu Lys Leu Glu Ile Gly Gly Gln
    210                 215                 220

Ser Leu Asn Leu Asp Cys Arg Glu Tyr Asn Ala Asp Lys Ala Ile Val
225                 230                 235                 240

Asp Ser Gly Thr Thr Leu Leu Arg Leu Pro Gln Lys Val Phe Asp Ala
                245                 250                 255

Val Val Glu Ala Val Ala Arg Ala Ser Leu Ile Pro Glu Phe Ser Asp
            260                 265                 270

Gly Phe Trp Thr Gly Ser Gln Leu Ala Cys Trp Thr Asn Ser Glu Thr
        275                 280                 285

Pro Trp Ser Tyr Phe Pro Lys Ile Ser Ile Tyr Leu Arg Asp Glu Asn
    290                 295                 300

Ser Ser Arg Ser Phe Arg Ile Thr Ile Leu Pro Gln Leu Tyr Ile Gln
305                 310                 315                 320

Pro Met Met Gly Ala Gly Leu Asn Tyr Glu Cys Tyr Arg Phe Gly Ile
                325                 330                 335

Ser Pro Ser Thr Asn Ala Leu Val Ile Gly Ala Thr Val Met Glu Gly
            340                 345                 350

Phe Tyr Val Ile Phe Asp Arg Ala Gln Lys Arg Val Gly Phe Ala Ala
        355                 360                 365

Ser Pro Cys Ala Glu Ile Ala Gly Ala Ala Val Ser Glu Ile Ser Gly
    370                 375                 380

Pro Phe Ser Thr Glu Asp Val Ala Ser Asn Cys Val Pro Ala Gln Ser
385                 390                 395                 400

Leu Ser Glu Pro Ile Leu Trp His His His His His His
                405                 410


<210> 69
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 69
Gly Leu Ala Leu Ala Leu Glu Pro
  1               5


<210> 70
<211> 8
<212> PRT
<213> Artificial Sequence
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<220>
<223> Description of Artificial Sequence: Peptide

<400> 70
Glu Val Lys Met Asp Ala Glu Phe
1 5

<210> 71
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 71
Glu Val Asn Leu Asp Ala Glu Phe
1 5

<210> 72
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 72
Leu Val Phe Phe Ala Glu Asp Val
1 5

<210> 73
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 73
Lys Leu Val Phe Phe Ala Glu Asp
1 5

<210> 74
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 74
cgctttaagc ttgccaccat gggcgcactg gcccgggcg 39

<210> 75
<211> 57
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 75
cgctttctcg agctaatggt gatggtgatg gtgccacaaa atgggctcgc tcaaaga 57

<210> 76
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 76
Arg Arg Gly Gly Val Val Ile Ala Thr Val Ile Val Gly Glu Arg
1 5 10 15